INVESTIGATING THE IMPACT OF ESSENTIAL OIL PRESERVATION SYSTEM IN THE DEVELOPMENT OF LIPID BASED NIACINAMIDE COSMECEUTICALS

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In partial fulfillment for the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

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October 2017

CERTIFICATE

This is to certify that the dissertation entitled **"INVESTIGATING THE IMPACT OFESSENTIAL OIL PRESERVATION SYSTEM IN THE DEVELOPMENT OF LIPID BASED NIACINAMIDE COSMECEUTICALS"** is a bonafide work submitted by **Reg. No.261511106,** to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfilment for **Master of Pharmacy** in **Pharmaceutics** and has been conducted under the guidance of **Dr. S. M. Habibur Rahman, M.Pharm, Ph.D.,** Department of Pharmaceutics, PSG College of Pharmacy, Peelamedu, Coimbatore in the academic year of 2016-2017(October 2017)

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DECLARATION

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EVALUTION CERTIFICATE

This is to certify that the dissertation work entitled **"INVESTIGATING THE IMPACT OFESSENTIAL OIL PRESERVATION SYSTEM IN THE DEVELOPMENT OF LIPID BASED NIACINAMIDE COSMECEUTICALS"** submitted by **University Reg. No. 261511106** to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by the candidate at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2016-2017.

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LIST OF ABBREVIATION

ABS	-	Absorbance
ADME	-	Absorption Distribution Metabolism Excretion
SLN	-	Solid Lipid Nanoparticle
NLCs	-	Nano Lipid Carriers
FT-IR	-	Fourier Transform Infrared
LFCS	-	Lipid Formulation Classification System
UV	-	Ultra Violet
SEM	-	Scanning Electron Microscopy
AFM	-	Atomic Force Microscopy
PCM	-	Phase contrast Microscopy

INTRODUCTION

LIPID BASED DRUG DELIVERY SYSTEMS

Drugs which are poorly water solubility are made well suitable for lipid-based formulation. Water insoluble and weakly basic drugs require special care in the design and development of lipid based formulation. These drugs administered in the solubilised form in the lipid vehicle may come out of the formulation due to solubilisation in the gastric fluid and may precipitate in the intestinal fluid on gastric emptying. The bioavailability of this system would depend on how rapidly the precipitates can be resolubilized by the formulation. (Sanjay Singh et al., 2009).

The percentage of new chemical entities synthesized with low aqueous solubility and high therapeutic efficacy is growing, this presents a major challenge for the drug delivery. To overcome the above challenge different methods were developed for the enhancement of bioavailability.

Lipid based formulations are more effective delivery system for oral route and improve bioavailability because of its proven safety and efficacy. Lipid Formulation Classification System was established by Pouton et al., It aims to enable *in vivo* studies for interpreting and for the identification of the most appropriate formulations for specific drugs, their physiochemical properties are taken into consideration. (Maulik Patel et al., 2011)

SOLID LIPID NANOPARTICLES (SLNs):

SLNs are particulate system with particle diameters ranging 50-1000nm. They are derived from oil-in-water emulsions, by replacing the liquid oil by a solid lipid. Particle size of SLN is in submicron range, ranging from 40 to 1000 nm. They have several advantages that the lipid matrix is generally made from physiologically well-tolerated lipid components, which decreases the toxicity. They have a stability of around 3 years and can easily be manufactured at industrial scales. SLNs, lipid micro particles and lipospheres have been used as alternative carriers for therapeutic peptides, proteins and antigens. Formulation as SLNs confers improved protein stability, avoids proteolysis, as well as providing sustained release of the incorporated molecules. Well-known peptides such as cyclosporine A, insulin, calcitonin and

somatostatin have been incorporated into solid lipid particles. (AlkaLohani, Anurag Verma et al., 2014).

NANO STRUCTURED LIPID CARRIER (NLC)

A Nano structured lipid carrier (NLC) are the new generation of lipid nanoparticles, attracting major attention as novel colloidal drug carriers which is composed of physiological lipid materials suitable for topical, dermal, and transdermal administrations. To illustrate, several problems have been reported with the conventional topical preparations, e.g., low uptake due to the barrier function of the stratum corneum and unwanted absorption to the systemic circulation. Several systems which are provided in literature review can deliver active pharmaceutical ingredients across the skin presenting advantages in systemic treatment with minimal side effects, the absence of first-pass metabolism, and in topical treatment allowing targeting specific skin appendages**.** NLC has been developed to overcome the drawbacks dealing with Solid Lipid Nanoparticles (SLN). SLN is produced by replacing the oil of an o/w emulsion by a solid lipid or a blend of solid lipid, i.e., the lipid particle matrix being solid at both room and body temperature. NLC consist mixture of solid lipid (long chain) and liquid lipid (short chain), preferably in a ratio of 70:30 up to a ratio of 99.9:0.1. The resulting matrix of the lipid particle shows a melting point depression compared to the original solid lipid, however, the matrix remains solid at body temperature. Some limitation of the SLN system regarding drug expulsion during storage, reduced particle concentration, reduced drug loading, these limitations were solved by formulating lipid particles with controlled nanostructure known as NLC. For some number of drugs, the solubility of liquid lipid is higher than that of solid lipid, which enhances drug-loading, NLC possess numerous features that are advantageous for the topical route of application. NLC are composed of physiological and biodegradable lipids that show low toxicity. The small size ensures a close contact to the stratum corneum and can increase the amount of drug penetrated into the skin. Due to the occlusive properties of lipid nanoparticles, an increased skin hydration effect is observed. Further these lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis. (shaileshpatwekar et al.,2014)

ADVANTAGES OF NLCs

- Their small size and relatively narrow size distribution permits site-specific drug delivery.

- Controlled and Sustained release of active drug can be achieved.
- The incorporated drug is protected from the onslaughts of biochemical degradation.
- High drug payload.
- Incorporation of lipophilic and hydrophilic drugs feasible.
- Can be sterilized by autoclave or gamma radiation.
- Can be lyophilizes and spray dried.
- Do not generate any toxic metabolites.
- Relatively cheap and stable.
- Ease of industrial scale production by hot dispersion technique.
- Surface modification can be easily performed.

PRODUCTION OF LIPID NANOPARTICULATE DRUG DELIVERY SYSTEM

There are two basic methods of SLN production: high pressure homogenization (HPH) and microemulsion technique. Also, attempts have been made to obtain those compounds using less expensive and complicated methods, such as ultrasound technique (US) and solvent cast method. Unfortunately, those methods have a number of disadvantages. (ElwiraLanson et al., 2011) The basic methods of obtaining solid lipid nanoparticles are outlined below.

High Pressure Homogenization (HPH)

HPH has proved to be an effective and reliable method of SLN production. Homogenizers of various sizes are available on the market for relatively favourable prices and HPH has been used in the production of nano-emulsions for a number of years. Contrary to other technique, HPH usually does not pose any difficulties for large-scale production. High-pressure homogenizers force the liquid through very thin orifices (a few microns in diameter) under the pressure of 100-2000 bar. On very short distances, the liquid reaches very high velocity of over 1000 km/h. High turbulence and shear disintegrate the particles to submicron sizes. The typical lipid content is 5÷10% and poses no difficulty for the homogenizer. Lipid nano-dispersion has been achieved with lipid concentration as high as 40%. HPH is further divided into hot and cold high pressure homogenization. In both methods the preparatory stage

involves the introduction of active substance into the lipids through dissolution or dispersion of those substances in liquefied lipid mass.

Hot homogenization technique:

Hot homogenization is conducted at temperatures higher than lipid melting point and can thus be considered homogenization in emulsion. Pre-emulsion of the active substance, melted lipid and water phase of the emulsifier is obtained in high speed mixer. The quality of pre-emulsions largely determines the quality of the end product. The desirable particle sizes are within a few micrometres. Hot homogenization of the pre-emulsion is conducted at temperature higher than lipid melting point. In general, the higher the temperature, the smaller the particle size, caused by viscosity reduction in the internal phase. However, too high temperature may cause the active substance and the carrier to decompose. The homogenization stage can be repeated a number of times. It should be noted that homogenization under increased pressure causes the emulsion temperature to rise by approx. 10° for every 500 bar. In most cases 3 to 5 homogenization cycles under 500-1,500 bar are sufficient. The increase of homogenization pressure or the number of cycles often results in the increase in particle size due to coalescence which is the product of high kinetic energy of particles. The basis product of hot homogenization is nanoemulsion in liquid state. Solid particles are obtained by cooling the sample to room temperature or lower. Due to small particle size and the presence of emulsifiers the crystallization of lipids can take very long (up to a few months).

Cold homogenization technique:

In the cold homogenization method, the lipid microparticles are obtained by melting and subsequent cooling of drug containing lipid followed by crushing, grounding and that is diffused in cold surfactant to obtain a cold pre-suspension of micronized lipid particles. This suspension is then passes through a high pressure homogenizer at room temperature by applying 5–10 cycles at 1500 bar. This method is most suitable for hydrophilic drugs with low solubility (surfactants are added to improve solubility). This technique shortens melting process of lipid and hence it is useful for thermo-sensitive and thermo-labile drugs.

Cold homogenization has been developed in order to overcome the three fundamental problems of hot homogenization:

- Decomposition of the active substance, caused by high temperature
- Decomposition of the active substance in the water phase duringhomogenization
- Complexity of the crystallization stage of the nanoemulsion, leading to multiplemodifications.

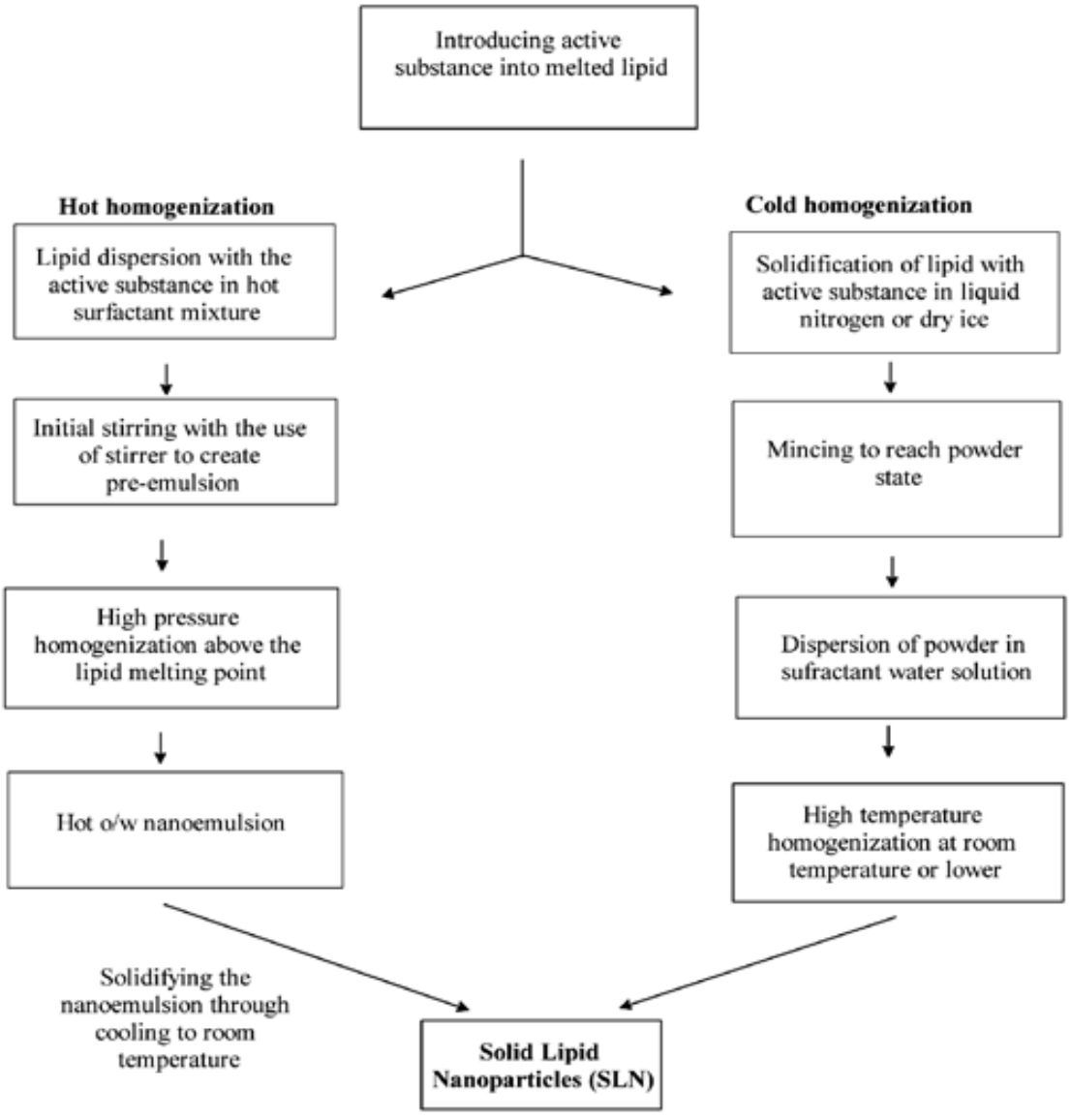


Fig 1: Schematic procedure of homogenization techniques for SLN production

Microemulsion technique

In order to obtain microemulsion with lipids in solid state at room temperature, the process temperature must be higher than lipid melting point. Lipids (e.g. fatty acids and/or

triglycerides) are melted and the mixture of water, emulsifiers and co-emulsifiers is heated to the temperature of the lipids and blended under mild conditions. If the procedure runs correctly, we will obtain transparent, thermodynamicallystable complex. The hot microemulsion is then dispersed in chilled water (2÷3°C) by smooth mechanical stirring, which ensures that the small particle size results from precipitation and not the mechanical stirring. The volume ratio of hot microemulsion to cold water should be from 1:25 to 1:50. The most popular emulsifiers are polysorbate 20, polysorbate 60 and soy lecithin. The most frequently used co-emulsifiers are usually alcohols, e.g. butanol. Technically, the precipitation of lipid particles in water is equivalent to diluting the complex, which leads to decrease in solid substance content in SLN dispersion. Due to diluting stage the achievable lipid content is lower than in formulations obtained through HPH. (ElwiraLanson et al.,2011).

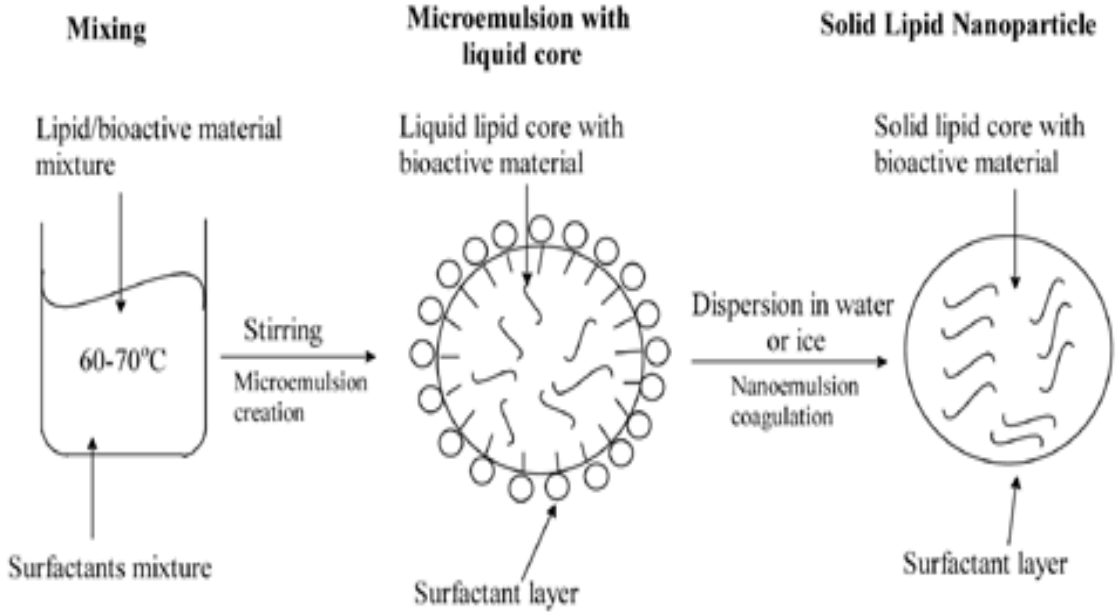


Fig 2: Schematic diagram showing the structures formed duringthe production of SLN by microemulsion technique

NUTRACEUTICALS

The quality of life in terms of income, spending and lifestyle has improved with economic Development. However, it has also thrown up a major challenge in the form of lifestyle Diseases'. The first victim of this lifestyle change has been food habits. Consumption of junk food has increased manifold, which has led to a number of diseases related to

nutritional deficiencies. Nutraceuticals can play an important role in controlling them. No wonder more and more people are turning to nutraceuticals. The term nutraceuticals was coined from nutrition and pharmaceutical in 1989by Stephen Defelice, founder and chairman of foundation for innovation in medicine, an American organization which encourages medical health [1, 2, 3, 4]. According to him “a nutraceutical is any substance that is a food or apart of food and provides medical or health benefits, including the prevention and treatment of disease”. Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods and herbal products. The concept of nutraceutical was stared from the survey in U.K., Germany and France and it concluded that diet is rated more highly by consumer then exercise or hereditary factors to achieving a good health. In the U.S. “nutraceutical"Was commonly used, but no regulatory definition existed. Its meaning was modified by health ministry of Canada which defines nutraceutical as “a product isolated or purified from the food, generally sold in medicinal form not associated with food and demonstrated to have a physiological benefit .It also provides benefit against chronic disease.

In Britain, the Ministry of Agriculture, Fisheries and Food has developed a definition of a functional food as "a food that has a component incorporated into it to give it a specific medical or physiological benefit, other than purely nutritional benefit [8].There is a slight difference between the functional foods and nutraceuticals. When food is being cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called “functional food". Thus, functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, etc. needed for its healthy survival. When functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) other than anemia, it is called a nutraceutical. Examples of nutraceuticals include fortified dairy products (e.g. milk) and citrus fruits (e.g. orange juice).

BENEFITS

• May increase the health value of our diet.

• May help us live longer.

• May help us to avoid particular medical conditions.

• May have a psychological benefit from doing something for oneself.

• May be perceived to be more "natural" than traditional medicine and less likely to produce unpleasant side-effects.

• May present food for populations with special needs (e.g. nutrient-dense foods for the elderly)

CLASSIFICATION OF NUTRACEUTICALS

Regarding the promise of nutraceuticals, they should be considered in two ways:

• Potential nutraceuticals

• Established nutraceuticals

A potential nutraceuticals is one that holds a promise of a particular health or medical benefit; such a potential nutraceuticals only becomes an established done after there are sufficient clinical data to demonstrate such a benefit. It is disappointing to note that the over whelming majority of nutraceuticals products are in the 'potential' category, waiting to become established. The food products used as nutraceuticals are categorized as

• Probiotic

• Prebiotic

• Dietary fiber

• Omega 3 fatty acid

• Antioxidant

ADVANTAGES OF ESSENTIAL OIL IN COSMETICEUTICAL FORMULATION

1. Enhancing the dermato-cosmetic properties and preservation, as well as the marketing image of the product.
2. At relatively high concentration in cosmetics it provides skin benefit.

NIACINAMIDE

Niacinamide is a derivative of vitamine B3 that suppresses melanin from reaching the surface of the skin and protects the skin from further UV damage. Niacinamide is a very effective skin-restoring ingredient that offers multiple benefits for aging skin, has been found to soothing activity which may be useful for blemished skin. Can improve the appearance of aged , hyperpigmented , and photodamaged skin. Niacinamide is categorized as BCS class I drugs owing to its poor aqueous solubility and poor GI absorption. In the present context, development of cosmeceutical with natural preservation system will be beneficial for the broad application and development of cosmeceutical. The present investigation is focused on

the development and optimization of natural preservation system and development of cosmetic loaded with nano lipid carrier with cosmeceutical.

OBJECTIVE

In Recent years, Bacterial contamination changes physical and chemical properties of cosmetics usually resulting in phase separation, discoloration and release of odours etc. Rich composition of modern cosmetics in combination with aqueous formulation and direct exposure to bacterial skin flora make them an ideal environment for microbial growth. Taking into consideration the high risk of contamination and therefore a risk for consumers health, the use of preservatives is a necessity.

Preservation systems prevent and control the growth of microorganisms from contamination during manufacturing, storage or consumer use. Completely preservative-free and microbial stable cosmetics are made by sterile production and appropriate packaging. However, satisfactory results can be achieved only for some formulations and are under certain restrictions.

Preservative systems usually include various combinations of chemical biocides that operate on a broad spectrum of bacteria and fungi. They offer a high antimicrobial efficacy and therefore prolong the shelf-life of products, however, many of them can cause adverse reactions to skin.

A promising strategy to overcome these problems involves the development of suitable drug carrier systems. Nowadays, essential oils are the subject of intensive scientific research and also attract attention of cosmetic and pharmaceutical industries due to their potential therapeutic benefits as well as natural preservation effect. A new promising field of application of essential oils as natural preservatives in cosmetics or feed additives in human or animal food or as plant protection products has been studied. It is estimated that more than 3000 essential oils are of commercial importance and used in flavor and cosmetic industries. The microbial safety of cosmetics has been always of special interest for industries, as microbial spoilage can lead to product degradation and cause a risk to consumer’s health.

It has become more and more evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. Exciting experimental data obtained *in vitro* are very often followed by disappointing results *in vivo* due to poor drug solubility, poor absorption, rapid metabolism and elimination, high fluctuation of plasma levels due to unpredictable bioavailability after per oral administration.

The carriers should permit a controlled and localized release of the drug according to the specific needs of the therapy which determines the *in vivo* fate of the drug. The size of the

carrier depends on the desired route of administration and ranges from few nanometres to the micrometer.

Nano structured lipid carriers are proved to be suitable carriers with various advantages like (i) controlled release of the drug (ii) increased drug stability (iii) high drug loading (iv) no bio toxicity of the carrier (v) avoidance of organic solvents and (vi) no problems with respect to large scale production and sterilization.

Based on these facts, the aim of this work is to present current knowledge on essential oils with special focus on mechanism of antimicrobial action; assessment of their efficacy as preservatives in cosmetic formulations as well as their safety is carried out with the following objectives.

- Selection of Natural Preservatives/ Oils
- Optimization of Natural Preservatives/ Oils in various Bacterial strains.
- Determine the Minimum inhibitory Concentration of essential oils.
- The specific objective of the present work is to develop nano structured lipid carriers (NLC's) loaded with Niacinamide using Natural Preservatives (Natural Essential Oils).
- To develop lipid based cosmeceuticals NLC using Niacinamide.
- To evaluate the physical and texture properties of the formulations.
- To characterize the prepared formulations.
- To evaluate the stability of the formulations over long term at room temperature.

REVIEW OF LITERATURE

NANO STRUCTURED LIPID CARRIERS; POTENTIAL DRUG CARRIERS

In the pharmaceutical breakthrough today, the new technologies lead to find numerous new mighty compounds. To double-check progress in drug therapy the development of new drugs solely is not sufficient. Poor water solubility and insufficient bioavailability of the new drug substances are very widespread issues encountered. Thus, there is an expanding need to develop a pharmaceutical carrier scheme that overcomes these matters. This carrier scheme should be free of toxicity, have an adequate pharmaceutical loading capability and the possibility of pharmaceutical targeting and controlled release characteristics. The system should provide chemical and personal steadiness for the incorporated pharmaceutical. The feasibility of the production technique and as well the affordability should also be accessible.

SLN have been presented as an alternate carrier scheme to emulsions, liposomes and polymeric nanoparticles. SLN are formulated from solid lipids only. Therefore, after groundwork at smallest a part of the particles crystallizes in a higher energy modification (α or β`). Throughout storage, these modifications can transform to the low power, more organised β modification. Due to this modification high degree of alignment, the number of imperfections in the crystal lattice is small; this directs to drug expulsion .NLC have been developed to overwhelm the drawbacks affiliated with SLN. They are advised to be the second lifetime of lipid nanoparticles. Contrasted to SLN, NLC show a higher loading capability for hardworking compounds by conceiving a less organized solid lipid matrix, i.e. by blending a fluid lipid with the solid lipid, a higher element drug stacking can be achieved.

NANO TECHNOLOGY AND NANO MEDICINE

Nanotechnology is extremely valuable in the development of advanced therapeutic systems, which are termed “nanomedicines”. In particular, drug delivery systems using nanoparticles area promising approach to improving the safety and bioavailability of drugs. Strongly lipophilic compounds are not generally applicable in aqueous biological systems. However, even if a drug is water insoluble, drug delivery materials permit its solubilization in the form of nanoparticle dispersions. Furthermore, targeted delivery of drugs to tissues and cells, as well as controlled release of the drug, are possible by varying the properties of these

nanoparticles.Thus, nanoparticulate drug delivery systems may lead to the development of novel therapeutic agents toward nanomedicine.

Approaches for the Development of Solid and Semi-Solid Lipid-Based Formulations

Lipid Based Drug Delivery (LBDD) has developed over the past decade fuelled by a better understanding of the multiple roles lipids may play in enhancing oral bioavailability. Moreover, the emergence of novel excipients with acceptable regulatory and safety profiles coupled with advances in formulation technologies have greatly improved the potential for successful lipid based formulations. With the growing interest in this field, there is an increasing need for guidelines in excipient selection and characterization; material handling, formulation design, and processing techniques to obtain effective and patient-compliant dosage forms. V. Jannin et al 2007 present the recent approaches in selecting the most appropriate lipid system(s); methods for characterization of their behaviour *in vitro* and *in vivo.*

Nutraceuticals

The term nutraceuticals was originally defined by Dr. Stephen L. DeFelice, founder and chairman of the Foundation of Innovation Medicine (FIM), Crawford, New Jersey. Since the term was coined by Dr.DeFelice, its meaning has been modified by Health Canada which defines nutraceuticals as: a product isolated or purified from foods, and generally sold in medicinal forms not usually associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease. Examples are beta carotene and lycopene. Dr Stephen DeFelice coined the term "Nutraceutical" from "Nutrition" and "Pharmaceutical" in 1989. The term nutraceutical is being commonly used in marketing but has no regulatory definition. An attempt to re-define nutraceuticals and functional foods is made in this article. The proposed definitions can help distinguish between functional foods, nutraceuticals, and dietary supplements. Nutraceuticals and dietary supplements. The advantages and disadvantages of nutraceuticals are also briefly discussed. Many nutraceuticals, functional foods and naturally occurring compounds that have been investigated and reported in various studies revealed that these products are extremely active, have profound effect on cell metabolism and often have little adverse effect. It is natural that people focus is shifting to positive approach for prevention of diseases to stay healthy. Nutraceuticals is scientific area generated all over the world. In many cases nutraceuticals off

advantage over the synthetic drugs under development by the pharmaceuticals industry. It is novel pharmacological activity that are become interesting in their possible clinical use and thus helping in prevention and therapeutic in several diseases.

Nutraceuticals is a broad umbrella term used to describe any product derived from food sources that provides extra health benefits in addition to the basic nutritional value found in foods. Products typically claim to There is minimal regulation over which products are allowed to display the nutraceuticals term on their labels. Because of this, the term is often used to market products with varying uses and effectiveness. The definition of nutraceuticals and related products often depend on the source. Members of the medical community desire that the nutraceuticals term be more clearly established in order to distinguish between the wide varieties of products out there. There are multiple different types of products that may fall under the category of nutraceuticals. Nutraceuticals have been claimed to have a physiological benefit or provide protection against the following diseases (and/or found to act as)

- Cardiovascular agents
- Antiobese agents
- Antidiabetics
- Anticancer agents
- Immune boosters
- Chronic inflammatory disorders
- Degenerative disease

Cosmeceuticals

Introduction

The word “cosmetic” is derived from the Greek word “Kosmos” meaning “to arrange”. In the late 10,000 BC, cosmetics were very important in Egyptian health and Hygiene. Tracing the origin of cosmetics, the first recorded use of cosmetic is attributed to Egyptians, Cirea 4000 BC.cosmeceuticals are cosmetic products with biologically active ingredients purporting to have medical or drug like benefits. A cosmeceutical is an ingredient with medicinal properties that manifests beneficial topical actions and provides protection against degenerative skin conditions. The word “cosmeceuticals” was popularized by Albert

M.Kligman in the late 1970s. It encompasses cosmetic actives with therapeutic, disease fighting, or healing properties, serving as a bridge between personal care products and pharmaceuticals ,like cosmetics , cosmeceuticals are topically applied ,but they contain ingredients that influence the biological function of skin.comeceuticals improve appearance by delivering nutrients necessary for healthy skin. Cosmeceuticals typically claim to improve skin tone, texture and radiance, while reducing wrinkling. Cosmeceuticals are the fastest-growing segment of the natural personal care industry.

Some of the other medication applications of the cosmeceutical products are:

• Anti-fungal & Anti-Bacterial

• Skin-Whitening Agent

• Anti-acne Agents

• Soothing, Smoothing, Moisturizing or Protective Agents (such as calamine)

• Under eye dark circle

• Anti-Ageing & Anti-Wrinkle

Niacinamide

Niacin is a water-soluble vitamine, also known as vitamine B3.niacin is the generic term for nicotinic acid (pyridine 3-carboxylic acid) and nicotinamide (nicotinic acid amide) and the coenzyme forms of the vitamine. Nicotinamide is active form, which functions as a constituents of two coenzymes, namely, nicotinamide adenine dinucleotide(NAD) and nicotinamide adenine dinuclotide phosphate(NADP). In the forms of these coenzymes, niacin functions in many biological redox reactions which activate about 200 dehydrogenase essential to electron transport and other cellular respiratory reactions.NAD functions as an electron carrier for intracellular respiration as well as a cofactor for enzymes involved in the oxidation (catabolism) of fats, protein, carbohydrates and alcohol to produce energy. NADP functions as a hydrogen donor in reductive biosynthesis(anabolism), such as in fatty acid and steroid synthesis. Like NAP, NADP is a cofactor for enzymes, such as in the oxidation of glucose-6-phosphate to ribose-5-phosphate in the pentose phosphate pathway.

In non-redox reactions NAD is the substrate for two classes of enzyme that separate the niacin moiety from NAD and transfer ADP –ribose to proteins. A third classes of enzymes catalyses the formation of cyclic ADP-ribose. This molecule also functions within cells to

provoke the release of calcium ions from internal storage sites and may also plays a role in cell-signaling.

Nano Structured lipid carriers (NLC)

Solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC) are colloidal lipid systems, which have been proposed for several administration routes, such as parenteral, oral and topical route providing controlled release profile of many substances. Solid lipid nanoparticle (SLN) has the chance to be exploited as a delivery system in commercial products. However, there are some limitations of the solid lipid nanoparticles (SLN) system: Drug expulsion phenomenon when lipid crystallizes to the stable β-form, particle concentration in the aqueous dispersions ranging from about 1% to a maximum of only 30% and limitation of drug load by the solubility of the drug in the solid lipid(A.C. Silva,Aakanchha Jain et al.,2011). These limitations were solved by creating a lipid particle with a controlled nanostructure i.e the nano structured lipid carrier (NLC).

SLN consists of pure solid lipids and NLC contains a certain percentage of additional liquid lipids leading to imperfections in the crystal lattice. These nanoparticles are produced by one of the following techniques, namely, high pressure homogenization, microemulsion template, cold homogenization, solvent emulsification, solvent diffusion, reverse micelle-double emulsion, homogenization followed by ultrasonication, solvent injection and a very recently introduced membrane contractor techniques(Amichand Dairam et al.,2008)

The suitability of solid lipid nanoparticles (SLN) for the encapsulation of lipophilic drug was assessed for oral administration. The hot high pressure homogenization (HPH) technique was used as production method for SLN. Mechanical approaches are capable of producing nanoparticles, typically in the 100–1000 nm range, whereas chemical methods tend to produce 10–100 nm particles.(A.C. Silva et al.,2011)

Nanotechnology is an enable technology that has the potential to revolutionize drug and food systems. Nanotechnology have shown enhanced oral bioavailability and biological efficacies of different phytochemicals(HuangQ et al. 2010)

ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS AND POTENTIAL APPLICATIONS IN FOOD

Essential oils are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production of EOs (Helander .I M et al.,1998).The term *'essential oil'* is thought to derive from the name coined in the 16th century by the Swiss reformer of medicine. An estimated 3000 EOs are known, of which about 300 are commercially important – destined chiefly for the flavours and fragrances market. It has long been recognised that some EOs have antibacterial properties and these have been reviewed in the past that spices have antibacterial properties. Besides antibacterial properties, EOs or their components have been shown to exhibit antiviral, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties.

The greatest use of EOs in the European Union (EU) is in food (as flavourings), perfumes (fragrances and aftershaves) and pharmaceuticals (for their functional properties). The well-known use of EO in aromatherapy constitutes little more than 2% of the total market. Individual components of EOs are also used as flavourings, either extracted from plant material or synthetically manufactured. The antibacterial properties of essential oils and their components are exploited in such diverse commercial products as dental root canal sealers, antiseptics and feed supplements for lactating sows and weaned piglets. A few food preservatives containing Eos (Sara Ann Burt et al.,2007).

Recently there has preservative and antimicrobial role of spices has been an increasing interest in discovering new natural prevention of meats. Bio preservatives include a range of natural plants, animals and microorganisms which can be used to improve the keeping quality of foods. Being plant natural food stuffs, spices appear to be an alternative for the chemical antimicrobials to the consumers who tend to question their safety. Spices active compounds have been included in class of naturally occurring food preservatives and their inclusion in foods allowed by food production regulatory offices. Although, spices have been well known for their medicinal, preservative and antioxidant properties, currently they have been used with primary purpose of enhancing the flavour of foods rather than extending shelf-life. In recent years antimicrobial properties of spices have been documented and interest continued to the present. There is little information available emphasizing the

preservative and antimicrobial role of spices in the prevention of meat (A.Jagadeesh Babu et al.,2011)

MECHANISM OF ANTIMICROBIAL ACTION OF ESSENTIAL OILS

Antimicrobial activities of EOs are well known and documented in numerous works. They are effective against both saprophytic bacteria and fungi, which are main source of cosmetic contaminations (*Bacillus* sp., *Micrococcus* sp., *Aereomonassp.*, *Acinetobacter* sp. and *Aspergillus* sp. or *Penicillium* sp.) and also against human pathogens (*Staphylococcus* sp., *Streptococcus* sp., *Salmonella* sp. or *Candida* sp. And others). In contrast to antimicrobial activity of EOs mechanisms of their action are still not fully understood and need elucidation. In general, antimicrobial activity of essential oils is determined by their composition and concentration of components. The number of constituents in essential oil can range from several up to far more than 100. Composition and proportion of compounds varies and depends on chemotype, age of a plant, climatic and environmental conditions as well as harvest time and the distillation method. Essential oils are a complex and diverse group of natural compounds that usually consist of terpenes with terpenoids, and also aromatic and aliphatic compounds of low molecular weight. Monoterpenes are the most commonly found molecules constituting 90% of essential oils in a great variety of structures. Aromatic compounds are represented less frequently, usually in trace amounts. EOs composition is often characterized by two or three main compounds at higher concentrations (20–70%), which determine biological properties of essential oil.

EFFICACY OF ESSENTIAL OILS AS PRESERVATIVES IN COSMETIC FORMULATIONS

Efficacy of preservatives in cosmetic formulations is evaluated in a challenge test according to the European Pharmacopoeia guidelines. The challenge test is a standard procedure that involves artificial contamination of cosmetics with predetermined number of bacteria and fungi (105-106 viable cells ml-1 or g-1 of product) as well as periodic removal of samples at fixed time for counting of viable microorganisms present in the formulation during test. Microorganisms used in the challenge test include strains of bacteria: *Staphyllococcus aureus*, *Pseudomonasaeruginosa*, *Escherichia coli* and fungi: *Aspergillus niger* and *Candida albicans*. According to EP, a topical preparation is well preserved if the number of the bacteria recovered per gram is reduced by a factor of 103 (criteria A) and 102 (criteria B)

within 2 days of the challenge test with no cell proliferation at 7th day up to the 28th day (Mariola Dreger, Karolina wiergus et al.,2013).

The present investigation is focused on the development of niacinamide loaded NLC and assessment of essential oil preservation system for cosmeceutical formulation containing niacinamide NLC.

PLAN OF WORK

- Preformulation Studies
  - Preparation of Calibration Curve for Niacinamide by UV Visible Spectrophotometric Analysis.
  - IR Spectroscopic Analysis.
  - Selection of Natural Preservatives/ Oils
  - Optimization of Natural Preservatives/ Oils in various Bacterial strains.
  - Determine the Minimum inhibitory Concentration of essential oils.

- Formulation Development
  - Formulation of nano structured lipid carrier using Ultra probe sonication technology.
  - Formulation of Base Cream.
  - Incorporation of NLC into Base.
- Characterization studies of the Niacinamide loaded NLCs Cosmeceutical
  - Particle Size Determination by Zeta sizer
  - Entrapment Efficiency
  - Microbial Studies
  - Atomic Force Microscopy(AFM)
  - Scanning Electron Microscopy(SEM)
  - Phase Contrast Microscopy(PCM)
  - *In vitro* skin penetration
  - Texture Analysis
  - Stability Studies as per ICH guidelines

MATERIALS USED

Table 1: Materials Used

SL.NO.	MATERIALS	SOURCE
1.	Niacinamide	Veer Chemie aromatics
2.	Saponin	Maddi Pharmaceuticals
3.	Compritol	Gattefosse
4.	Soya lecithin	Loba Chemie Pvt. Ltd., Mumbai
5.	Glyceryl mono stearate	Loba Chemie Pvt. Ltd., Mumbai
6.	Cocoa Butter	National chemicals,vadodara,Gujarat
7.	Cinnamon oil pure	Sangrose Laboratories Pvt. Ltd., Mavelikara
8.	Distilled Water	Himedia Lab., Mumbai
9.	Muller Hinton Agar	Himedia Lab., Mumbai

EQUIPMENTS USED

Table 2: Equipments Used

SL.NO.	EQUIPMENT	MODEL/COMPANY
1.	Digital Weighing Balance	Shimadzu AY 220
2.	Magnetic Stirrer	Remi Equipments Ltd
3.	Bath Sonicator	RP 120 Ralsonics, Mumbai
4.	Ultra Probe Sonicator	SONICS vibracell
5.	UV Visible Spectrophotometer	UV-1650 PC Shimadzu
6.	FT IR Spectrophotometer	8400 S Shimadzu
7.	IR Hydraulic Pellet Press	Model M15 Technosearch Instruments
8.	ELIZA reader	Thermo Scientific
9.	Scanning Electron Microscope	JEOL, Japan- JSM 6360
10.	Atomic Force Microscope	Multimode Scanning probe microscope (NTMDT, NTEGRA prima, Russia)
11.	Zeta Sizer Nano ZS90	Malvern UK
12.	Texture Analyzer	TA-XT Plus
13.	Phase Contrast Microscope	NIKON Inverted Fluorescent Microscope

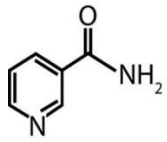
DRUG PROFILE

NIACINAMIDE

Chemical name : pyridine-3-carboxamide

Formula : C6H6N2O

Structure :



Molecular weight :122.127g/mol

Physical state : Solid

Melting point : 130 °C

Solubility : Water solubility 83.1mg/ml .
Very soluble in ethanol , slightly soluble in chloroform.

Density : 1.4 g/cm^3

Log P : 0.29

Log S : -0.17

Pka : 3.35(at 20 °C)

Hydrogen acceptor count : 3

Hydrogen donor : 1

Pharmacological actions : It has got many potent pharmacological actions like antipruritic, antimicrobial, vasoactive, photo-protective, sebostatic and lightening effects depending on its concentration. niacinamide controls the NFκB-mediated transcription of signalling molecules by inhibiting the nuclear poly (ADP-ribose) polymerase-1 (PARP-1).

EXCIPIENTS PROFILE

COMPRITOL

Synonyms	**:**	glyceryl behenate
Chemical Name	**:**	2,3-dihydroxy propyl docoanoate.
Empirical Formula	**:**	$C_{25}H_{50}O_4$
Molecular Weight	**:**	414.671g/ml
Functional Category	**:**	Emulsifying agent; solubilising agent; tablet and capsule lubricant.
Applications	**:**	Glyceryl behenate is a fat used in cosmetics, foods, and oral pharmaceutical formulations. In cosmetics, it is mainly used as a viscosity-increasing agent in emulsions.
Description	**:**	Compritol® 888 ATO is an inert, tasteless/odorless, hydrophobic white powder used in formulation of solid oral dosage forms..
Melting point	**:**	65–77°C
Solubility	**:**	Glyceryl bebenate is soluble when heated, in chloroform and dichlromethane. It is practically insoluble in ethanol(95%), hexane, mineral oil and water

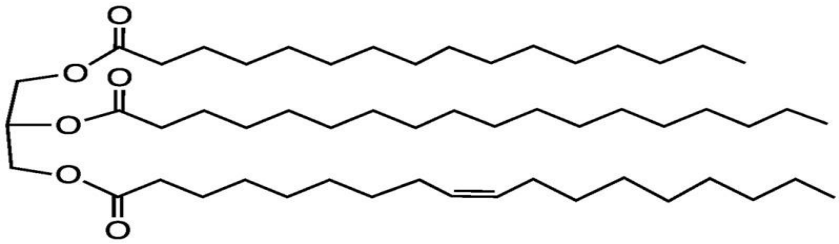
COCOA BUTTER

Synonyms : Cocoa oil, Cocoa fat, Cocoa bean oil

Chemical name : 1,3-dipalmitoyl- 2-oleoyl-glycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS), and 1,3-stearoyl-2-oleoyl-glycerol

Empirical formula : $CH_3(CH_2)_{16}COOH$

Structural formula :



Description : Yellow in colour

Melting point : 34.1°C (93.4° F)

Solidify : Solidify at 20°C

Refractive index : 1.44556- 1.44573

Iodine value : 32.11- 35.12

Acid value : 1.68

Saponification value : 191.214

Functional category : It is used in lotions and skin care products like cream, facial washes, etc.,

GLYCERYL MONOSTEARATE

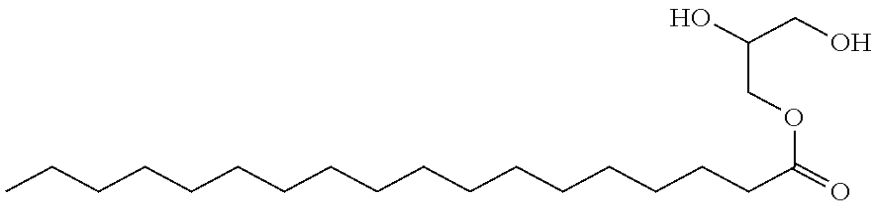
Synonym : Glyceryl monostearate, Glycerin monostearate, Monostearin

Chemical name : 2,3-Dihydroxypropyl octadecanoate

Empirical formula : $C_{21}H_{42}O_4$

Molecular weight : 358.56 $g \cdot mol^{-1}$

Structural formula :



Description : A white or yellowish white, hard waxy mass or unctuous powder orflakes; odourless or slight, agreeable, fatty odour.

Boiling point : 238 to 239 °C

Melting point : 58 to 59 °C

Functional category : GMS is a food additive used as a thickening, emulsifying, anti-caking, and preservative agent; an emulsifying agent for oils, waxes, and solvents; a protective coating for hygroscopic powders; a solidifier and control release agent in pharmaceuticals; and a resin lubricant. It is also used in cosmetics and hair care products.It is responsible for giving ice cream and whipped cream its smooth texture. It is sometimes used as an anti-staling agent in bread.

Storage : Glyceryl monostearate should be kept in a tightly closed container, protected from light.

CINNAMON OIL

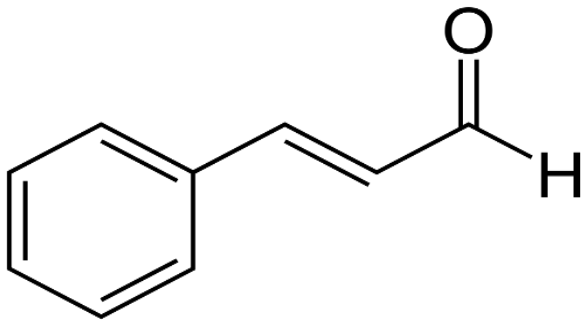
Synonym **:** Cassia oil, Chinese cinnamon,Cassia bark oil, Oil of cassia

Chemical name **:** 2-methoxy-4-prop-2-enylphenol;[(E)-prop-1-enyl]benzene

Empirical formula **:** $C_{19}H_{22}O_2$

Molecular weight **:** 282.383 g/mol

Structural formula **:**



Description **:** It is of a golden-yellow colour, with the characteristic odour of cinnamon and a very hot aromatic taste. The pungent and scent come from cinnamaldehyde (about 90% of the essential oil from the bark) and, by reaction with oxygen as it ages, it darkens in colour and forms resinous compounds.

Boiling point **:** 194-234 °C

Solubility **:** Insoluble in water, soluble in alcohol

Functional category **:** Effective at treating skin conditions such as rashes, acne and infections, you can mix cinnamon essential oil with a carrieroil (like coconut oil) and apply it to the skin to take advantage of its antimicrobial capacity.

PREFORMULATION STUDIES

Preformulation studies involve physical, chemical and biological characterization of new drug substances in order to develop stable, safe and effective dosage form. Preformulation testing encompasses all studies enacted on a new drug compound in order to produce useful information for subsequent formulation of a stable and bio-pharmaceutically suitable drug dosage form.

Analytical methods for Niacinamide

- UV Spectrophotometric estimation of Niacinamide (European Pharmacopoeia 6.0)

Determination of lambda max:

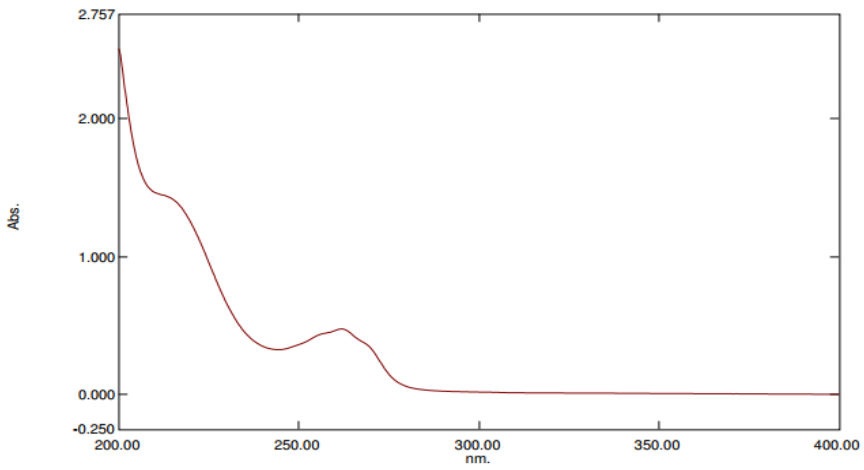
Niacinamide:

A concentration of 100µg/ml solution of Niacinamide, were prepared and scanned under UV Visible Spectrophotometer from 200 to 400nm. The corresponding peak with the highest absorbance was taken as the lambda max for quantifying Niacinamide.

Niacinamide standard solution (100µg/ml) showed highest peak at 261nm. From the UV-Vis spectrum (Graph 1) the lambda max of the Niacinamide was optimized as 261nm and used for further studies.

Lambda max of Niacinamide:

Fig 3 : UV Spectrum of Niacinamide



No.	P/V	Wavelength	Abs.	Description
1	↑	261.80	0.475	
2	↓	319.40	0.010	
3	↓	244.20	0.325	

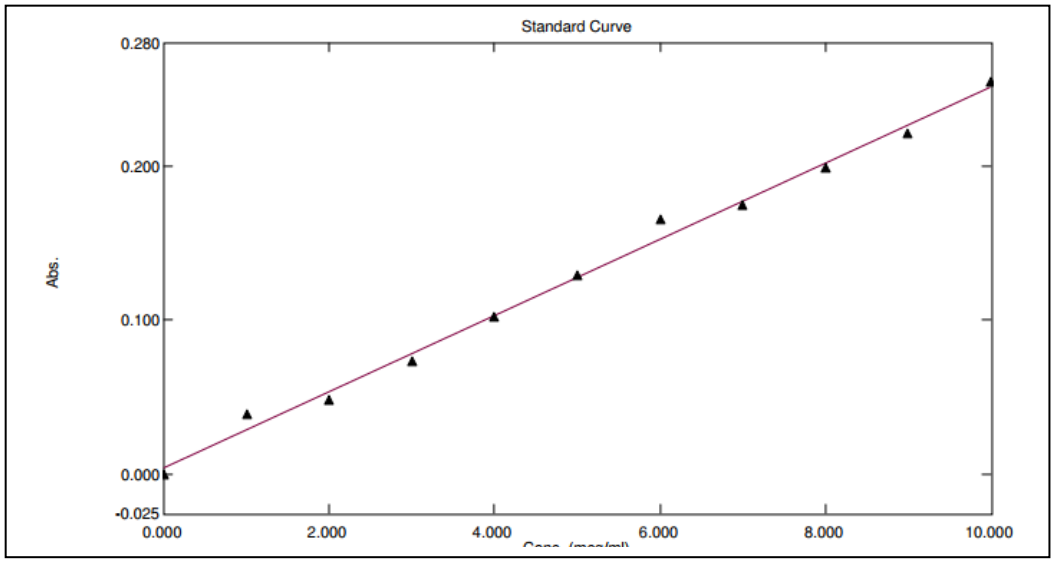
Standard graph for Niacinamide

Primary stock solution of Niacinamide was prepared by dissolving 100mg of Niacinamide in 100ml of methanol in a volumetric flask. Aliquots of Niacinamide was prepared from stock solution in the concentration range of 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml, 5 µg/ml, 6 µg/ml, 7 µg/ml, 8 µg/ml, 9 µg/ml and 10 µg/ml in 100ml volumetric flask using water as solvent. The absorbance of Niacinamide standard solutions was measured at 261 nm (lambda max of Niacinamide) against water as blank. The standard graph was prepared with concentration of solution (in µg/ml) on X-axis and absorbance on Y-axis. The results are shown in fig 4.

Table 3: Standard Table for Niacinamide

Sl.no	Concentration (µg/ml)	Absorbance at 262nm
1.	1	0.039
2.	2	0.048
3.	3	0.073
4.	4	0.102
5.	5	0.129
6.	6	0.166
7.	7	0.175
8.	8	0.199
9.	9	0.222
10.	10	0.255

Fig 4: Calibration Curve of Niacinamide



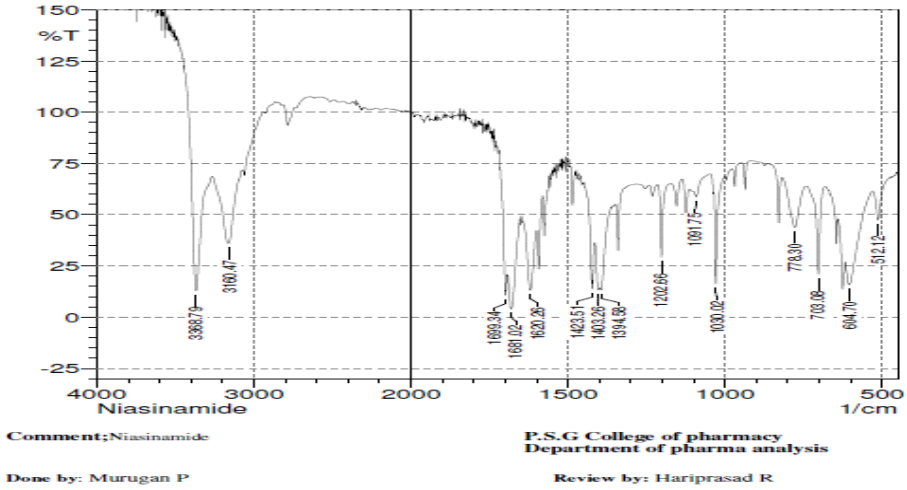
Compatibility studies

The sample of Niacinamide of about 10mg was mixed with 100mg of KBr to make the pellet and scanned under FT-IR spectroscopy from 400 – 4000 cm-^{1}.

IR Spectrum of Niacinamide

Characteristic peaks for carbonyl group C=O stretching was observed in 1681 cm-^{1}and N-H Amide Stretching at 3368 cm-^{1} which is evident that two peaks are confirming the purity of Niacinamide molecule.

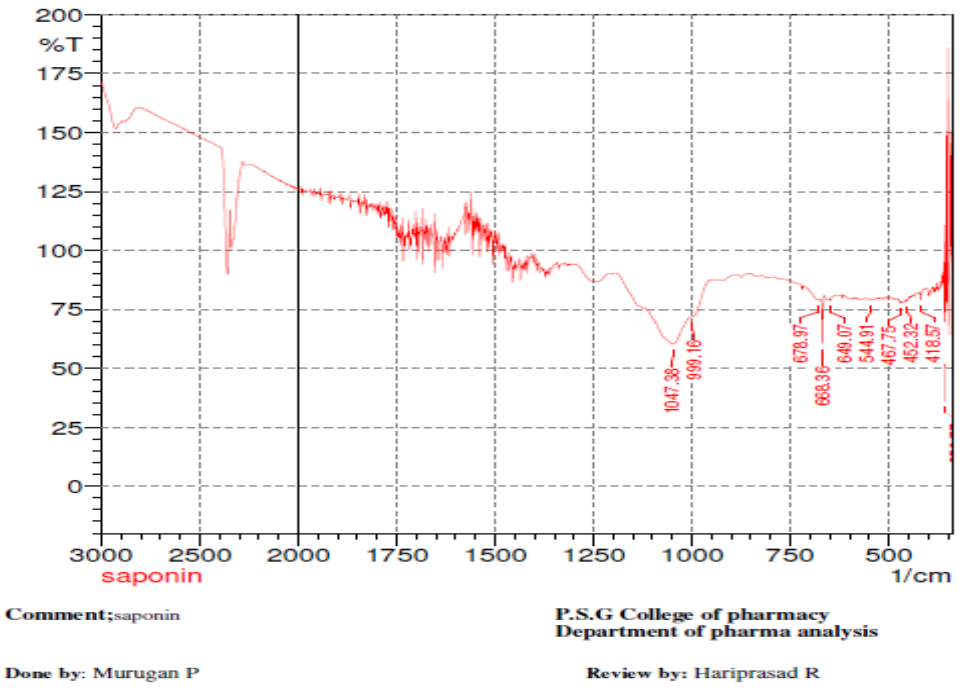
Fig 5 : IR Spectra of Niacinamide



IR Spectrum of Saponin

Characteristic peaks for carbonyl group O-CH3 stretching was observed in 1047 $cm^{-1}$and C-H bond with benzene Stretching at 678 cm^{-1} and sharp peak indicate geometrical cis-isomer stretching was observed in 999 cm^{-1} which is evident that three peaks are confirming the purity of saponin molecule.

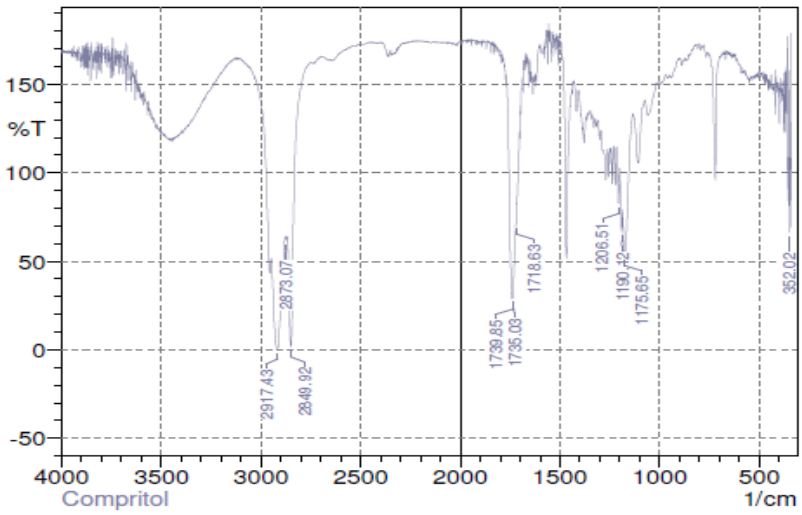
Fig 6 : IR Spectra of Saponin



IR Spectrum of compritol

Characteristic peaks for carbonyl group C=O stretching was observed in 1739 $cm^{-1}$and C-H Stretching at 2849 cm^{-1} which is evident that two peaks are confirming the purity of compritol molecule.

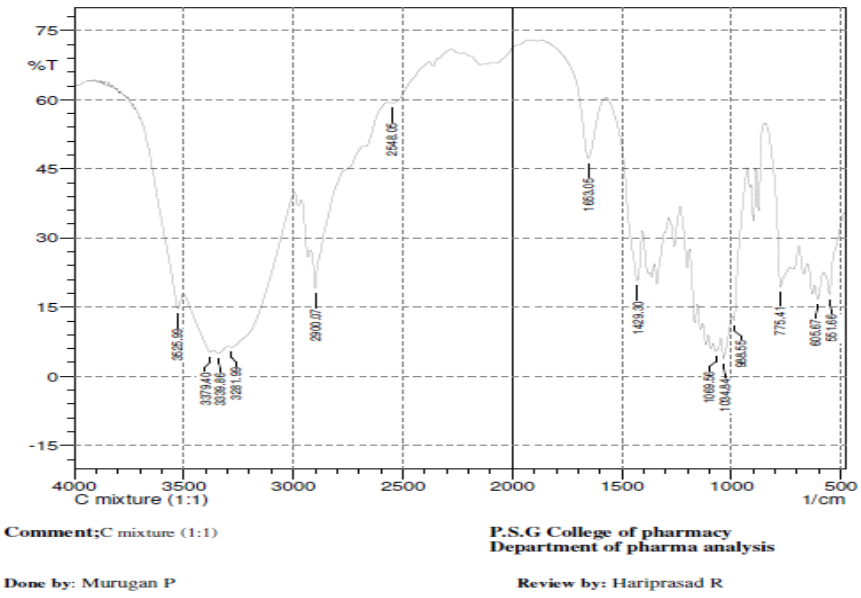
Fig 7 : IR Spectra of Compritol



IR spectra of physical mixture niacinamide, saponin and compritol

Characteristic peaks for carbonyl group C=O stretching was observed in 1653 cm-^{1}and C-H Stretching at 2900 cm-^{1} and N-H amide Stretching at 3379 cm-^{1} and C-H bond with benzene Stretching at 998 cm-^{1} which is evident that four peaks are confirming the purity of physical mixture niacinamide, saponin and compritol molecule.

Fig 8 : IR spectra of physical mixture niacinamide, saponin and compritol



PREPARATION OF PLANT MATERIAL FOR SAPONIN DETERMINATION

The rinds of healthy ripe fruits of Sapindus mukorossi were sun dried. The dried samples were then crushed with mortar and pestle before grinding into fine powder using a manual grinder.

QUALITATIVE DETERMINATION OF SAPONIN

The homogenous samples of sapindus mukorossi was subjected to phytochemical analysis for qualitative determination of saponin .The performed qualitative tests were briefly described as:

In a test tube 0.5g og the extract was shaken with water. A stable frothing was taken as a evidence for the presences of saponin.

The following ranking was used;

+ = Present

QUANTITATIVE DETERMINATION OF SAPONIN

Air-dried powdered pericarp of Sapindus mukorossi(5g) was extracted by maceration in methanol at 50°C for 12hours at a solid/liquid ratio of 1/20(w/v).After filtration and evaporation of the methanol extract, the gummy residue was suspended in 50mL water and extracted successively with 100mL ethyl ether three times. After removing the remaining ethyl ether in the aqueous layer by evaporation, the solution was further extracted successively with 100mL, and 50mL n-butanol respectively, then, the n-butanol fraction was dried by evaporation. The resulting product (1.57g) is referred to as the organic extract of crude saponin.

The saponin content was calculated by using the following formula.

Percentage of saponin= (final weight of sample/initial weight of extraction) ×100

Percentage of saponin= (1.57/5) ×100

Percentage of saponin=31.4%

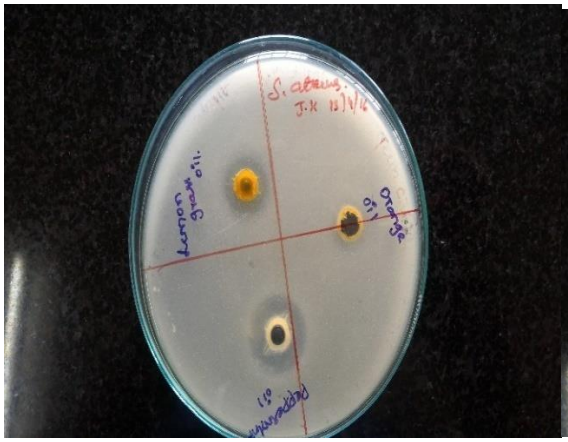
SELECTION OF OILS

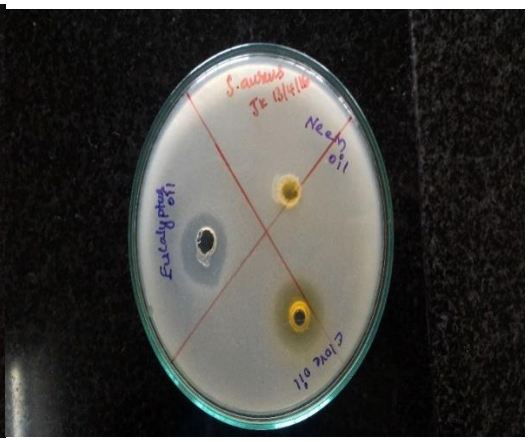
Various Essential oils have been widely used for bactericidal, virucidal, fungicidal, ant parasitical, insecticidal, and other medicinal properties such as analgesic, sedative, anti-inflammatory, spasmolytic, and locally anesthetic remedies. Essential oils such as Neem

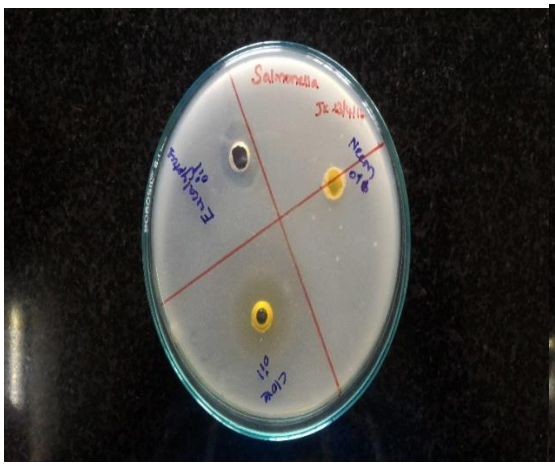
oil,Clove oil,Eucalyptus oil,Orange oil,Peppermint Oil,Lemon grass Oil,Castor oil,Lavender Oil, Olive oil, Cinnamon oil tested for antibacterial activity against 7 Bacterial Strains. (Staphylococcus aureus, Enterococcus faecalis, Streptococcus aeruginosa, Salmonella typhimurium, E.coli, Klebseilla pneumonia,Candida albicans).

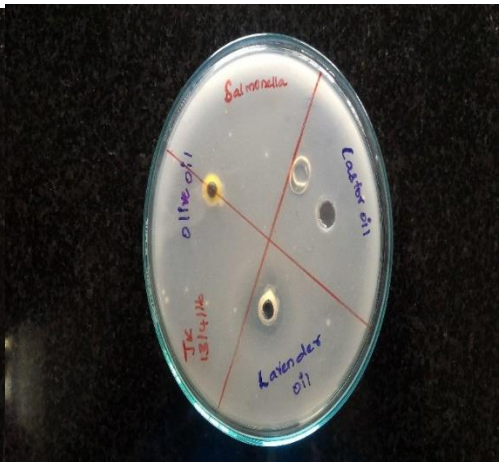
Various Essential oils	**Strains**						
	Staphylococcus Aureus	**Solmonella typhimurium**	**Enterococcus faccalis**	**Pseudimonas aeroginosa**	**E.coli**	**Klebsiella pneumonia**	**Candida albicans**
Neem oil	-	-	-	-	-	-	-
Clove Oil	✓	✓	-	✓	✓	✓	-
Eucalyptus Oil	✓	✓	-	✓	✓	✓	-
Orange Oil	-	-	-	-	-	-	-
Peppermint Oil	✓	✓	-	✓	✓	✓	-
Lemon Grass Oil	-	✓	-	✓	✓	✓	✓
Castor Oil	-	-	-	-	-	-	-
Lavender Oil	✓	✓	-	✓	✓	✓	✓
Olive Oil	-	-	-	-	-	-	-

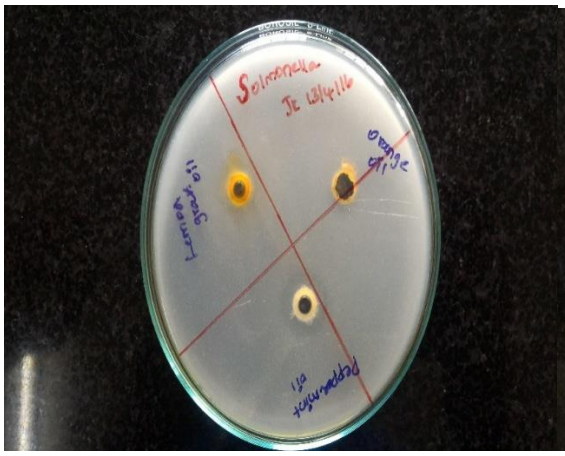
Table 4:Antibacterial activity of Essential Oils

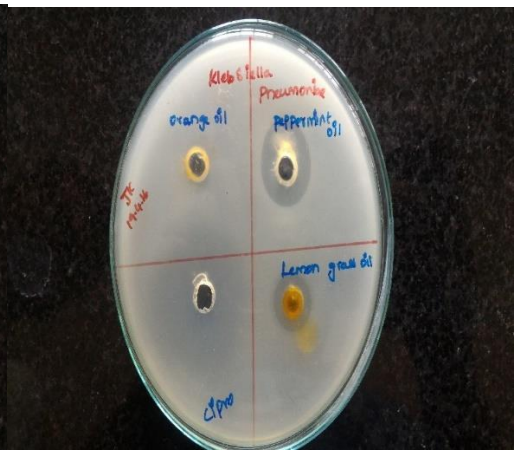

S. aureus
J.K 13/4/16
Lemon grass oil
Orange oil
Peppermint oil

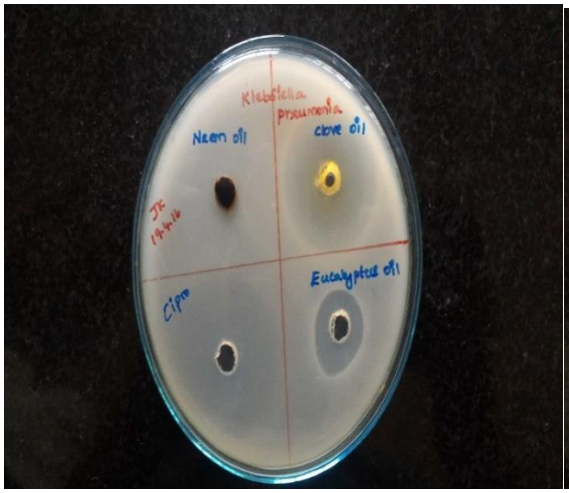

S. aureus
Je 13/4/16
Neem oil
Eucalyptus oil
Clove oil


Salmonella
Je 13/4/16
Neem oil
Eucalyptus oil
Clove oil


Salmonella
Olive oil
Castor oil
Je 13/4/16
Lavender oil


Salmonella
Je 13/4/16
Orange oil
Lemon grass oil
Peppermint oil


Klebsiella Pneumoniae
orange oil
Peppermint oil
Je 13/4/16
Lemon grass oil
Clove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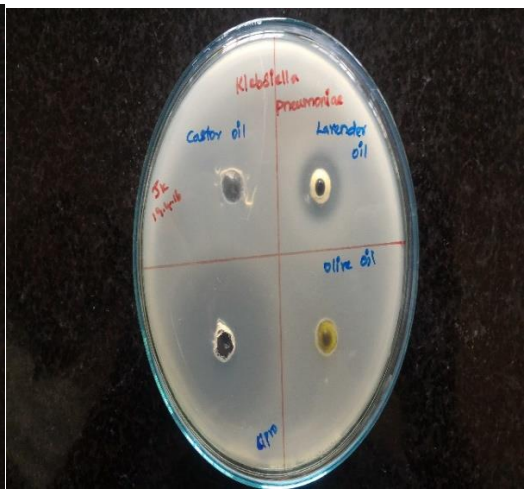
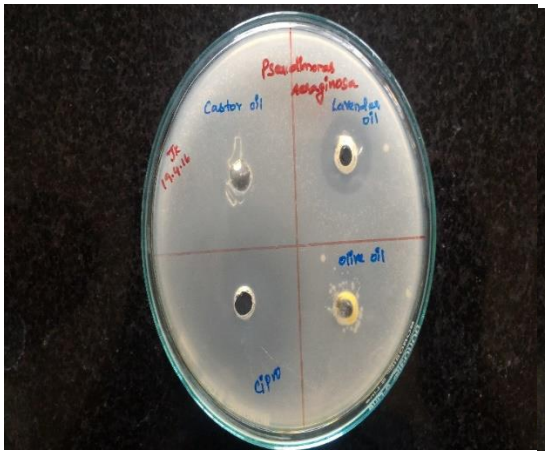
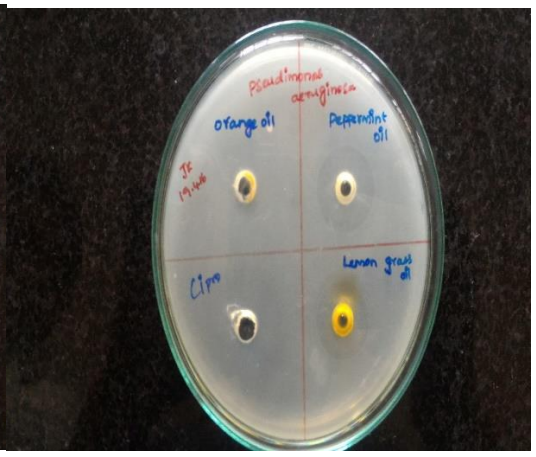
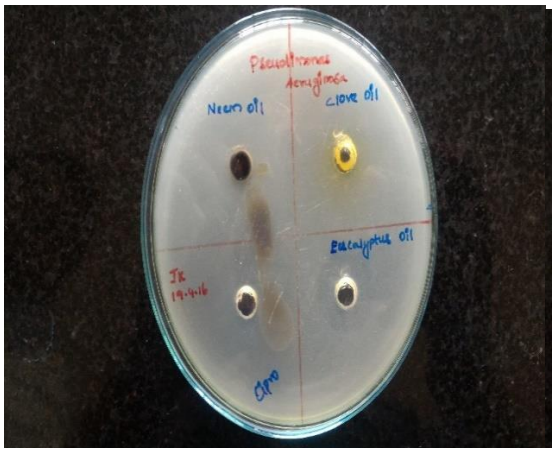
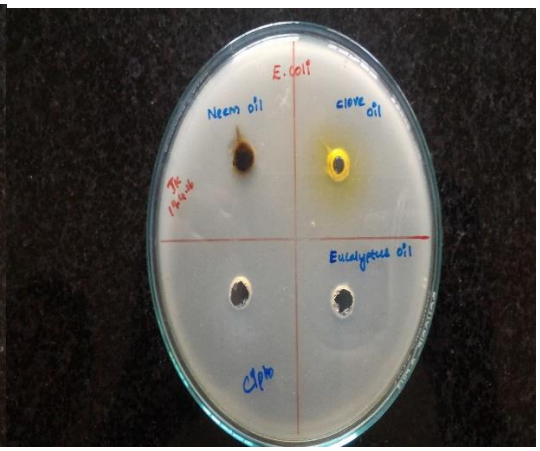
clove oil
Eucalyptus oil
Cipro


Klebsiella pneumoniae
Castor oil
Lavender oil
olive oil
Cipro


Pseudomonas Aeruginosa
Castor oil
Lavender oil
olive oil
Cipro


Pseudomonas aeruginosa
Orange oil
Peppermint oil
Lemon grass oil
Cipro


Pseudomonas Aeruginosa
Neem oil
clove oil
Eucalyptus oil
Cipro


E. Coli
Neem oil
clove oil
Eucalyptus oil
Cipro

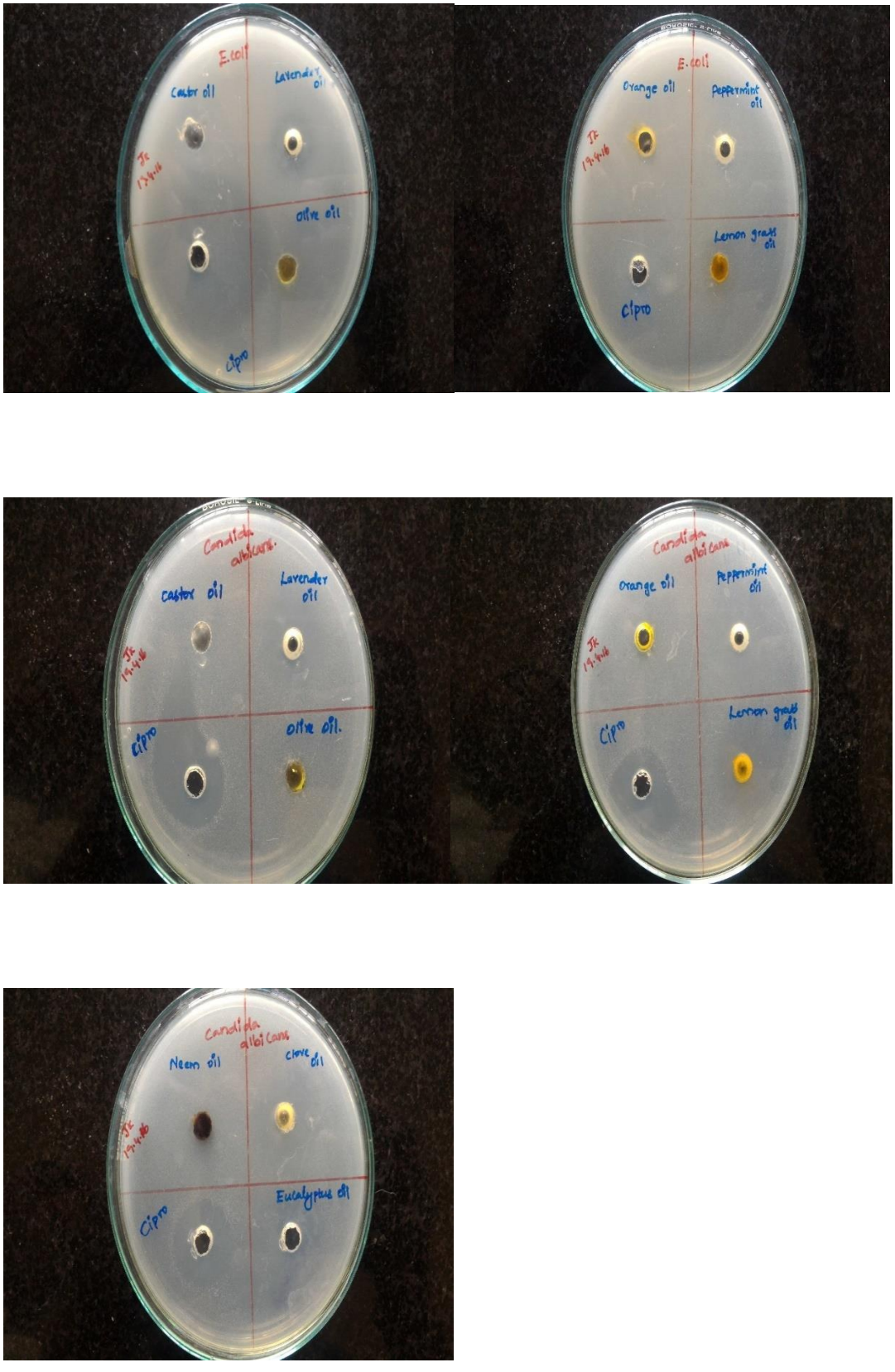


Fig 9 : Images Of Zone Of Inhibition For Essential Oils

Among this clove oil, peppermint oil, lemon grass oil, lavender oil, Cinnamon oil have well effect against bacteria.Neem oil has less bacterial activity presents in the active constituent of neem bark oil other than that it has fungal activity.

Mixed proportions of essential oils were studied for antimicrobial activity for E.Coli and Klebsiella strains

Clove Oil + Eucalyptus Oil	Clove Oil + Lavender Oil
Clove Oil + Peppermint Oil	Eucalyptus Oil + Peppermint Oil
Peppermint Oil + Lavender Oil	Clove Oil + Eucalyptus Oil + Peppermint Oil
Clove Oil + Eucalyptus Oil + Peppermint Oil + Lavender Oil	Clove Oil + Eucalyptus Oil + Peppermint Oil + Neem Oil
Clove Oil + Eucalyptus Oil + Peppermint Oil + Lavender Oil + Neem Oil	Cinnamon Oil + Neem Oil

Table 5: Mixed Proportion Of Essential Oils

Fig 10: Images of Zone of Inhibition For Mixed Proportion

Here combination of oils shows lesser antibacterial activity than individual.

DETERMINATION OF MINIMUM INHIBITORY CONCERNTRATION

Method: 1

MIC determinations were performed in 96-wellmicroplates according to procedures described by the Clinical and Laboratory Standards Institute. The liquid media was prepared by adding 2% peptone, 0.5% Nacl, 0.3% beef extract in 100ml distilled water. Then 100µl liquid media was transferred into micro plate well. 100µl of essential oil were added in first well then serial two fold dilution was made. Add 5µl inoculum in each well. The positive control comprised of media and organism and the negative control was liquid media. Inoculated micro plate were incubated for 24hrs at 37°C. Then absorbance were measured spectrometrically at 625 nm.

Control	**Absorbance**
Media	0.0470
Media + Organism	0.1156

Essential oil	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Cinnamon oil	0.1956	0.4966	0.4074	0.2406	0.0921	0.0913	0.0777	0.0843	0.0751	0.0928	0.0711
Eucalyptus oil	0.0568	0.0791	0.0736	0.0768	0.0786	0.0999	0.1606	0.1432	0.1417	0.1314	0.1382

Peppermint oil	0.6197	0.1587	0.0968	0.0962	0.0902	0.1201	0.1071	0.1225	0.1244	0.1443	0.1291
Lavender oil	0.2285	0.1144	0.1425	0.0783	0.1368	0.1970	0.1752	0.2272	0.1981	0.2594	0.1268

Table 6: Minimum inhibitory concentration for essential oils

Method: 2

All microbiological assays were performed under anaerobicconditions. MIC determinations were performed in 96-wellmicroplates according to procedures described by the Clinical and Laboratory Standards Institute. Each essential oil (200mg) was dissolved in dimethyl sulfoxide (40µL) and thevolume was made to 5 mL with sterile Muller Hinton medium containing 1%Tween 80 to provide a stock solution containing 40 mg $mL^{-1}$of oil. Serial twofold dilutions of each essential oil stockwere made with Muller Hinton medium to yield final concentrations ranging from 20 to 0.625 mg $mL^{-1.}$ The diluted samples (100 µL)were transferred to microplate wells and mixed well withthe micropipette. The negative controls comprised sterile Muller Hinton medium or with dimethyl sulfoxide (at concentrations usedin the dilutions). In order to ascertain aseptic conditions, the control wells contained sterile Muller Hinton medium but withoutinoculum. The inoculated microplates were incubated at36 ± 1◦C for 48h under anaerobic conditions; and the bacterial growth was confirmed by adding 10µL of a sterile0.5% aqueous solution of triphenyltetrazolium chloride (TTC,Sigma–Aldrich) and incubating at 36◦C for 30min. Theviable bacterial cells reduced the yellow TTC to pink/red1,3,5-triphenylformazan (TPF). All assays were performed intriplicate.

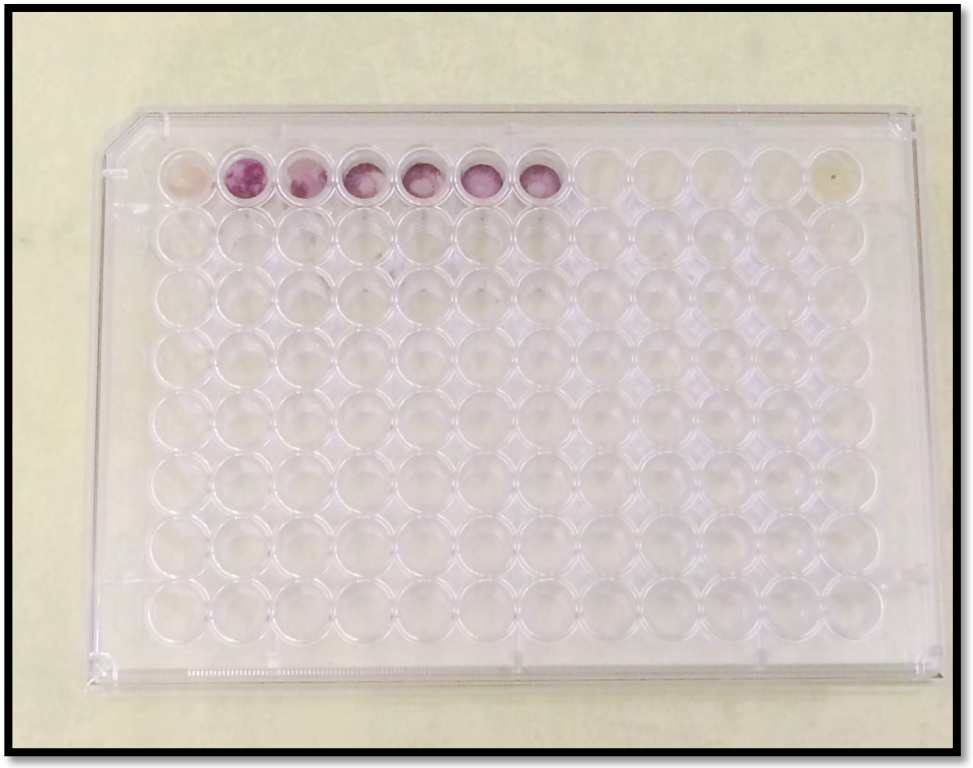


Fig 11: Minimum InhibitoryConcentration for Peppermint Oil

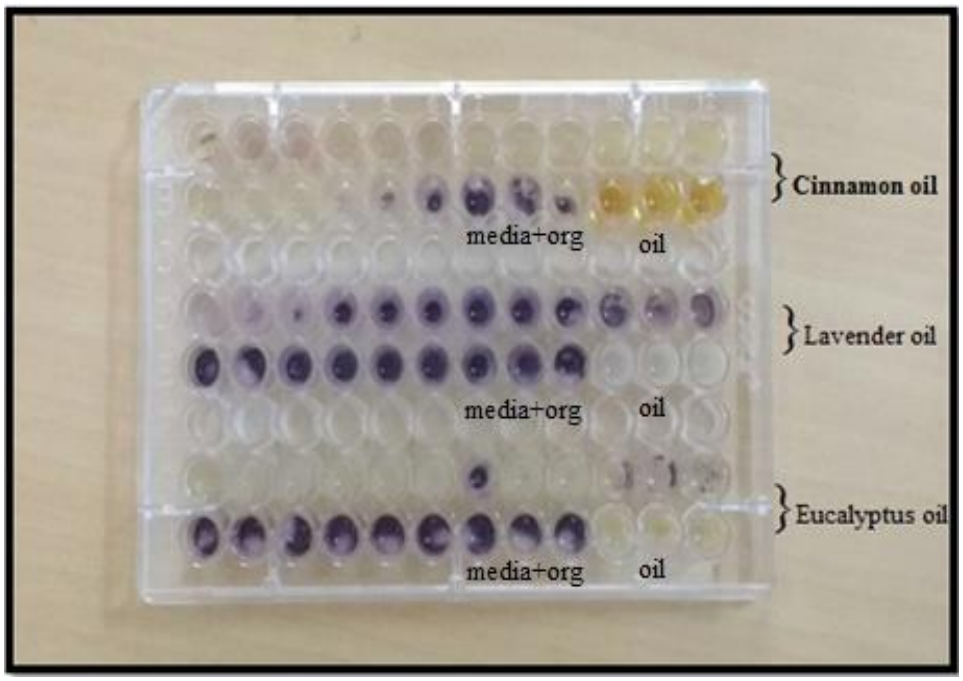


Fig12: Minimum Inhibitory Concentration for Cinnamon Oil, Lavender Oil And Eucalyptus Oil

EXPERIMENTAL METHODOLOGY

Nanostructured lipid carriers (NLCs) are the second Generation solid lipid nano particles (SLN) composed of solid lipid matrix which are incorporated with liquid lipids. Among the nanostructured lipid carriers that contain solid lipids together with different liquid oils .The presence of liquid lipids with different fatty acid C-chains produces NLCs with less organized crystalline structure and therefore provides better loading capacity for drug accommodation. Liquid lipids are better solubilizers of drugs than solid lipids.

Approaching nano structured lipid carrier development from an emulsion perspective is faced with significant challenges. Numerous research groups subsequently commenced research efforts to improve nano structured lipid carrier development. Most researchers have approached traditional emulsion techniques.

MATERIALS

Saponin(sapindusmukorossi),Comprittol,CocoaButter,Glycerylmonostearate, Soya lecithin,Cinnamon oil.

FORMULATION DEVELOPMENT

Preparation of Nano Structured Lipid Carriers (NLCs) by High Shear Homogenization coupled with ultra probe sonication

The solid lipid(1000mg) of choice and the liquid lipid (0.1ml)was mixed with the drug (50mg) and warmed to 75°C for effective melting and mixing. Simultaneously, distilled water(20ml) to which the surfactant(200mg) has been incorporated is also heated to 75°C,it is instilled into the formulation here in. Thereafter, the aqueous part is added to the lipid part maintaining the temperature at 75°C, with continous stirring followed by magnetic stirring for 20mins. The two- phase system is then sonication using probe sonicator, at 20,000rpm for 10min followed by ultra sonication for 2 min. The prepared formulations are stored at refrigeration condition until further use.

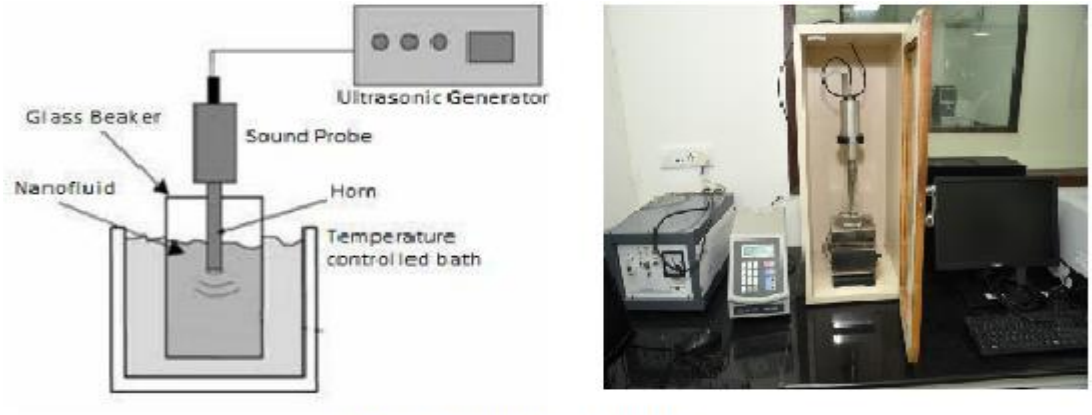


Fig 13 :Schematic representation of the configuration of a Ultra Probe Sonicator

Preparation of Base Cream:

In this study, O/W emulsion was prepared by the addition of aqueous phase to the oily phase with continuous agitation. To prepare the base, an lipid phase that consisted of cocoa butter (20gm), glyceryl monostearte (2.5gm), and cinnamon oil(0.3ml). At the same time, aqueous phase consisting of distilled water (50ml), soya lecithin (3.5gm), was heated to the same temperature. After heating, aqueous phase was added to the lipid phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for 15 min until complete aqueous phase was added. After the complete addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization,for a period of 5 min, and then the speed of the mixer was reduced to 500 rpm for further 5 min for complete homogenization, until the emulsion cooled into room temperature.

Incorporation of NLCs into Base Cream

Prepared Niacinamide loaded NLCs formulation (20ml) was incorporated into the 20g of Base Cream.

CHARACTERIZATION STUDIES

Particle size analysis

- ***Photon correlation spectroscopy:***

The prepared NLCs dispersions were diluted with water /suitable solvent and the sample were analyzed for particle size by photon correlation spectroscopy technique using Zeta sizer (Nano ZS 90), Malvern, UK.

- ***Zeta potential***

The size distribution and the charge nature of the prepared solid lipid Nano particle loaded with cosmaceuticals was analyze using Malvern zeta seizer. The suitable dilutions of the dispersions were made using water and it was scanned under version 6.30 by using disposable sizing cuvette at the count rate of 317.5 kcps for 60 sec at the measurement position of 4.6mm at attenuator 10.

Encapsulation efficiency:

2ml of the formulation was taken and subjected to centrifugation at 13000 rpm for 50 min at 4°c. The supernatant was collected and the absorbance was measured at the corresponding lamda max of 261nm.

ANTIMICROBIAL ASSAY

Nutrient agar was prepared and poured on petriplates for the growth of bacterial cultures. The Test cultures such as *Escherichia coli (Gram –ve), Staphylococcus aureus (Gram +ve)* was swapping on the plates by swape method. The prepared formulation were made on sterile disc (20µg/ml) was added and drying in the incubator for one day. Then the disc was placed in agar plate and standard as control. Bacterial test culture plates were incubated at 32-37°c for 24 hours. The sensitivity of test organism to each formulations were indicated by clear zone of inhibition around the well and the diameter of the zone of inhibition was measured.

Atomic Force Microscopy

A small aqueous drop of the Niacinamide loaded nanoparticles was adsorbed and dried to the surface of glass slide at room temperature. The images were examined on Multimode Scanning probe microscope (NTMDT, NTEGRA prima, Russia) in semi-contact mode with a force constant range of 0.35- 6.06 N/m and a resonating frequency range of 47- 150 KHz. The phase image and topology image were used to determine the morphology of the NLCs.

Scanning electron microscopy

Scanning electron microscope (SEM) is a type of electronmicroscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The surface characteristics of prepared NLCs were examined by scanning electron microscope (SEM). The suspension was first put on clear glass stub, allowed to dry in air followed by coating with gold using Polaren E 5100 sputter coater and observed under microscope at 5.5x magnification.

***In vitro* drug release studies:**

NLCs formulation 1ml was taken in Franz diffusion cell apparatus. A Pig earskin was placed in a Frans diffusion cell containing water as medium at room temperature. At various predetermined intervals i.e., 2hr, 4hr, 6hr, 8hr, 24hr, 48hr. 1ml of samples was withdrawn and replaced by water, to maintain the sink conditions. Cumulative % drug release in the samples were determined by measuring the absorbance under UV visible spectrophotometry and thereby extrapolating from the calibration curves.

Texture analysis:

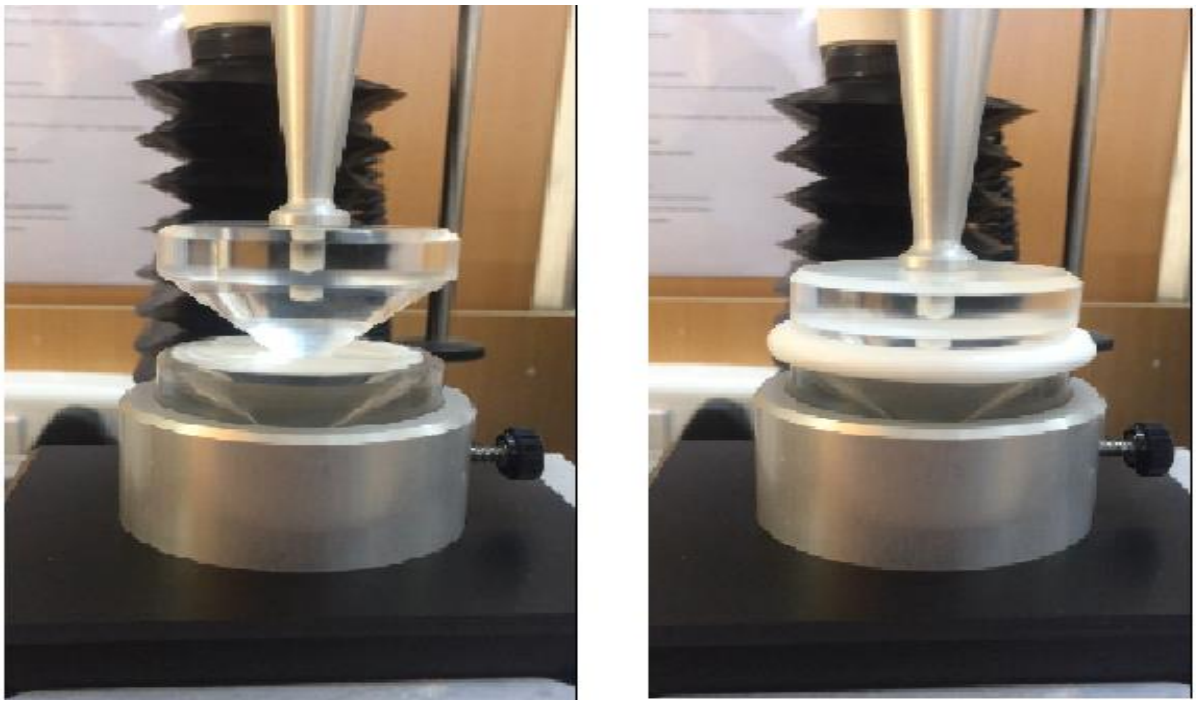


Fig 14: Texture Analysis Image for Niacinamide loaded Cream

Determination Of Spreadability Of Cream:

The spreadability of the cream was determined by means of Texture Analyser (TA.XT plus) equipped with 5 kg load cell using spredability rig as fixture (figure). This fixture consists of a heavy duty platform, male cone and a female cone. The Heavy duty platform was placed on the base of the machine and locked in the desiredposition by tightening the screws. An empty femalecone sample holder was placed in the base holder. The male cone probe was attached above the female cone such that the male conefits almost all the wayinto the female cone sample holder and proper care is taken to align the cones in this position. The height of the male cone was calibrated against thefemale cone so that the starting point was 25.0mm above the female cone (2 mm form the tip of the male cone and the sample). After calibration the sample was placed in the female cone sample holder and the test was run. The values of firmness (g) and work of shear (g s) were noted down by running macros.

Determination of Bloom Strength

The bloom strength of the cream was determined by means of Texture Analyser (TA.XT plus) equipped with 5 kg load cell using a cylindrical probe of 0.5'' diameter as fixture (figure). The sample in the container was placed centrally on the platform beneath the cylindrical probe. After calibrating the height of the probe, the test was commenced. A trigger force of 10 g was used for the study. The test results are obtaining by running the macro.

Stability studies:

Particle size analysis and entrapment efficiency studies were conducted for 3 month to evaluate the stability of the formulations. The stability studies were carried out according to ICH guidelines. The NLCs formulations was stored at two different conditions i.e, 5 ± 3˚C (refrigeration conditions) and 25 ± 2˚C (room temperature).

RESULTS AND DISCUSSION

The profound success of lipid based formulations for highly potent, lipophilic drug molecules have gained focus in this research field from the perspective of pharmaceutical industries. To increase the success rate of these lipids based formulations, there is a need to understand the excipients role. In this line, the present study is carried out to develop nano structured lipid carrier using the selected lipid with surfactants.

FORMULATION DEVELOPMENT

- **Selection of Lipid**

The lipid Compritol was selected randomly based on the stability data of the previously formulated Niacinamide solid lipid nanoparticles. The lipid showed high drug encapsulation with good stability was selected for further studies.

Preparation of Nano Structured Lipid Carrier

Ultra probe sonicator was used to formulate NLCs of Niacinamide. Totally, 7 formulations were prepared using one Solid lipid (Compritol), one Liquid lipid (Cinnamon oil), and surfactants (Saponin, Soya Lecithin). The formulae for the prepared NLCs were given in the table No 7.

Table 7: Batch specification of Niacinamide loaded NLCs

Formulation Code	Drug (mg)	Solid Lipid (mg)	Liquid Lipid (ml)	Surfactant1 (mg)	Surfactant2 (mg)	Water (ml)	Remarks
NLCN1	50mg	500mg	0.5ml	50mg	-	20ml	Phase inversion
NNLC2	50mg	500mg	0.5ml	100mg	-	20ml	*Unstable
NNLC3	50mg	500mg	0.5ml	150mg	-	20ml	Creaming on storage
NNLC4	50mg	1000mg	0.5ml	200mg	-	20ml	Good emulsion
NNLC5	50mg	50mg	0.5ml	200mg	-	20ml	Good emulsion
NNLC6	1000mg	200mg	0.5ml	200mg	50mg	20ml	Creaming on storage
NNLC7	1000mg	2000mg	0.5ml	200mg	1000mg	20ml	Creaming on storage

***Product is not processed for further studies due to stability issues**

Fig 15: Formulation of Niacinamide loaded NLC

PHYSICOCHEMICAL CHARACTERIZATION

Particle Size Analysis

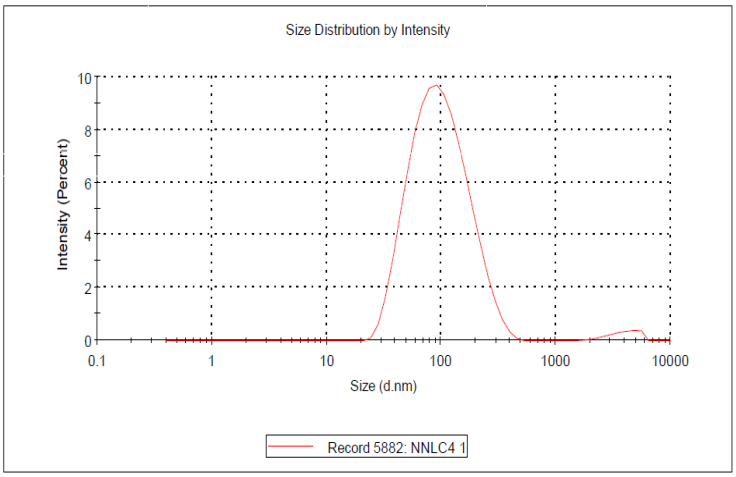
The prepared nano structured lipid carrier was subjected to particle size analysis using Zeta sizer (nano ZS90, Malvern, UK). The formulations were sufficiently diluted with double distilled water prior to the measurement. The results showed that the particle size of prepared formulations were in the range of 83 to 686nm with good PDI.

The results suggest that the incorporation of different surfactant showed a difference in the aggregation pattern of prepared particles. This may be due to the difference in the solubility of lipid in surfactants

Table 8: Particle size measurement results of Niacinamide loaded NLCs

Formulation Code	Particle size (nm)	Zeta potencial (mV)	PDI
NNLC 1	373.5	- 5. 84	0.575
NNLC 3	420	0.189	10,000
NNLC 4	**83.33**	**- 3.52**	**0.257**
NNLC 5	162.3	- 6.5	0.525
NNLC 6	686	- 10.7	0.931
NNLC 7	71.31	- 7.15	0.413

Size distribution report by intensity



Zeta potential report

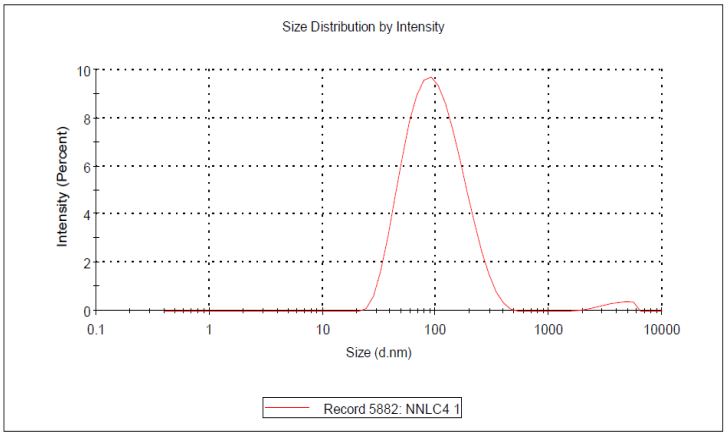


Fig 16: Zeta size analysis of Niacinamide loaded NLCs prepared using Compritol, saponin and 0.1ml Cinnamon oil

Entrapment efficiency

The drug loading into the nanoparticles was determined by subjecting the formulations to centrifugation at 13000 rpm for 50 mins and supernatant was separated. The amount of free drug in the supernatant was measured spectrophotometrically at 261nm. A high drug loading was observed for all the formulations. Drug payload of 89.88% was observed for the formulation NNLC 4. The results are depicted in table 9.

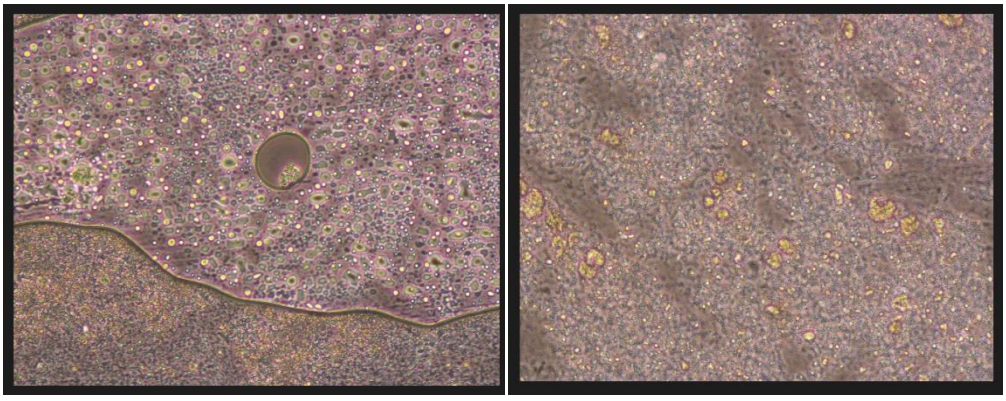
Table 9: Percentage entrapment of drug in NLCs

Formulation	Entrapment Efficiency (%)
NNLC 1	83.23
NNLC 3	57.19
NNLC 4	89.88
NNLC 5	84.81
NNLC 6	81.54
NNLC 7	80.20

Phase Contrast Microscopy

The Phase Contrast Microscope images for Niacinamide loaded NLCs formulation, Formulation loaded cream shows particles are uniformly dispersed and it has spherical shape.

Fig 17: PCM images showing the morphology of Niacinamide loaded NLCs



Niacinamide NNLC 4 – 40X

Anti microbial activity

The present study deals with the preliminary screening and comparison of Antimicrobial activity of prepared formulation of NLCs loaded Niacinamide. The prepared formulations have been tested against *E.coli* and *S. aureus* for antimicrobial activity. The antimicrobial activity is more in NNLC 4 against *E.coli*, *S. aureus*. The antimicrobial activity of NLCs was compared against standard (Gentamycin) as control.

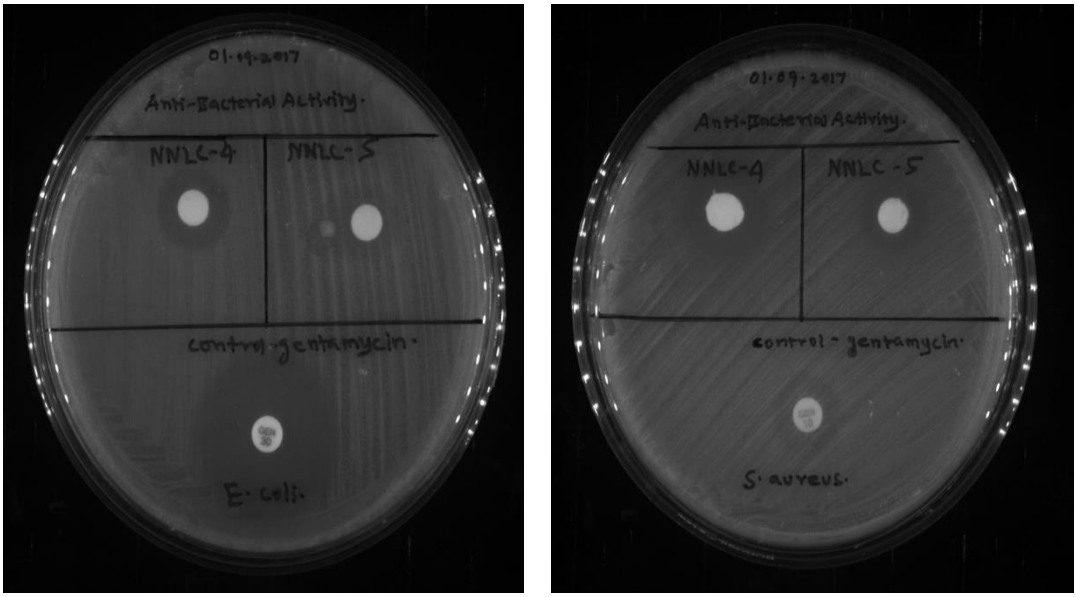


Fig 18: Anti microbial activity Images of prepared NLCs

Table 10: Antimicrobial activity of niacin loaded NLCs

S.no	Bacterial strains	Zone of Inhibition (mm)		
		NNLC 4	NNLC 5	Control
1	*E.coli*	14	10	29
2	*Staphylococcus aureus*	20	14	17

SCANING ELECTRON MICROSCOPY

The surface characteristics of prepared NLCs were examined by scanning electron microscope (SEM). The suspension was first put on clear glass stub, allowed to dry in air followed by coating with gold using Polaren E 5100 sputter coater and observed under microscope at different magnification.

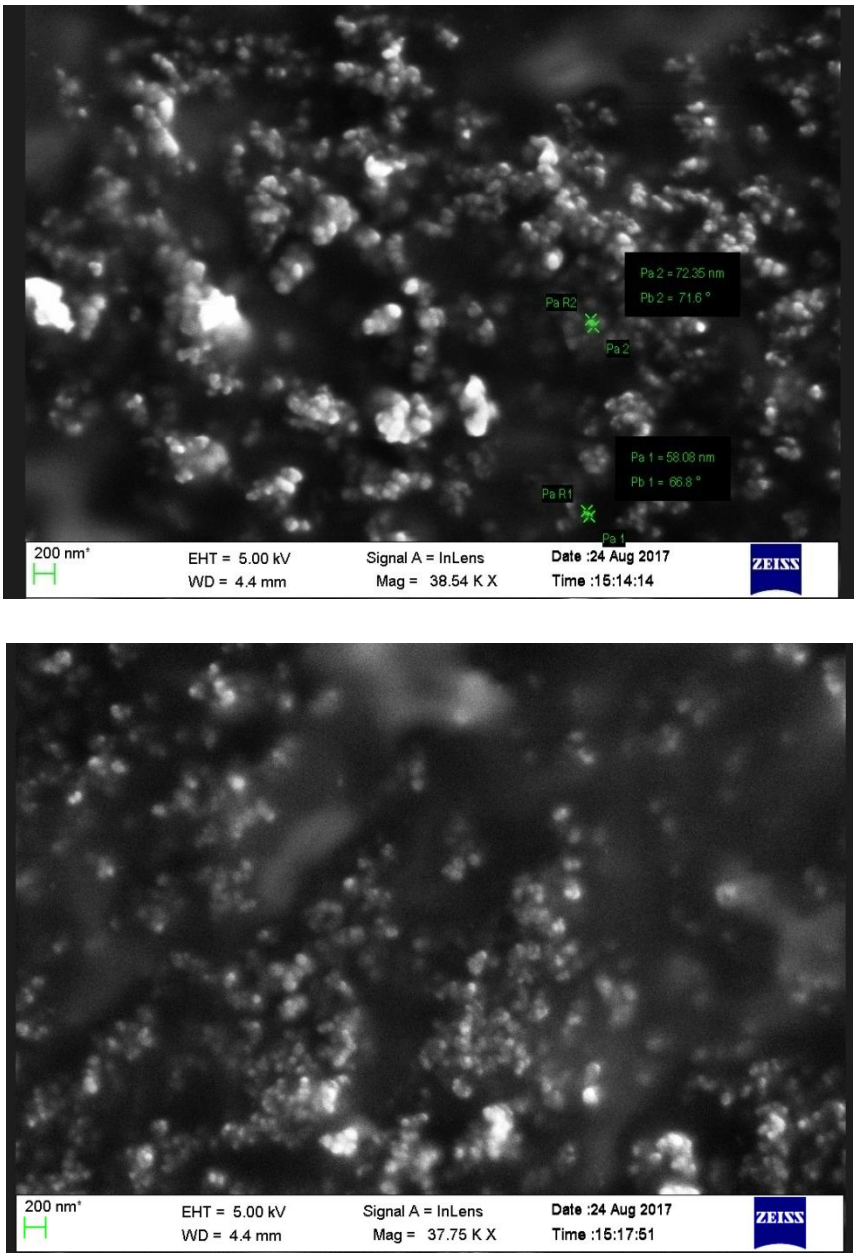


Fig 19: SEM Images of prepared NLC

Scanning electron micrographs of NLCs are shown in the above Figures. The shape of the NLCs was spherical and the size of the NLCs was found within the nanometer range. Moreover, the micrograph also revealed the agglomeration of nanoparticles which might be

due to the lipid nature of the carrier and the drying process during sample preparation prior to SEM analysis.

ATOMIC FORCE MICROSCOPY (AFM)

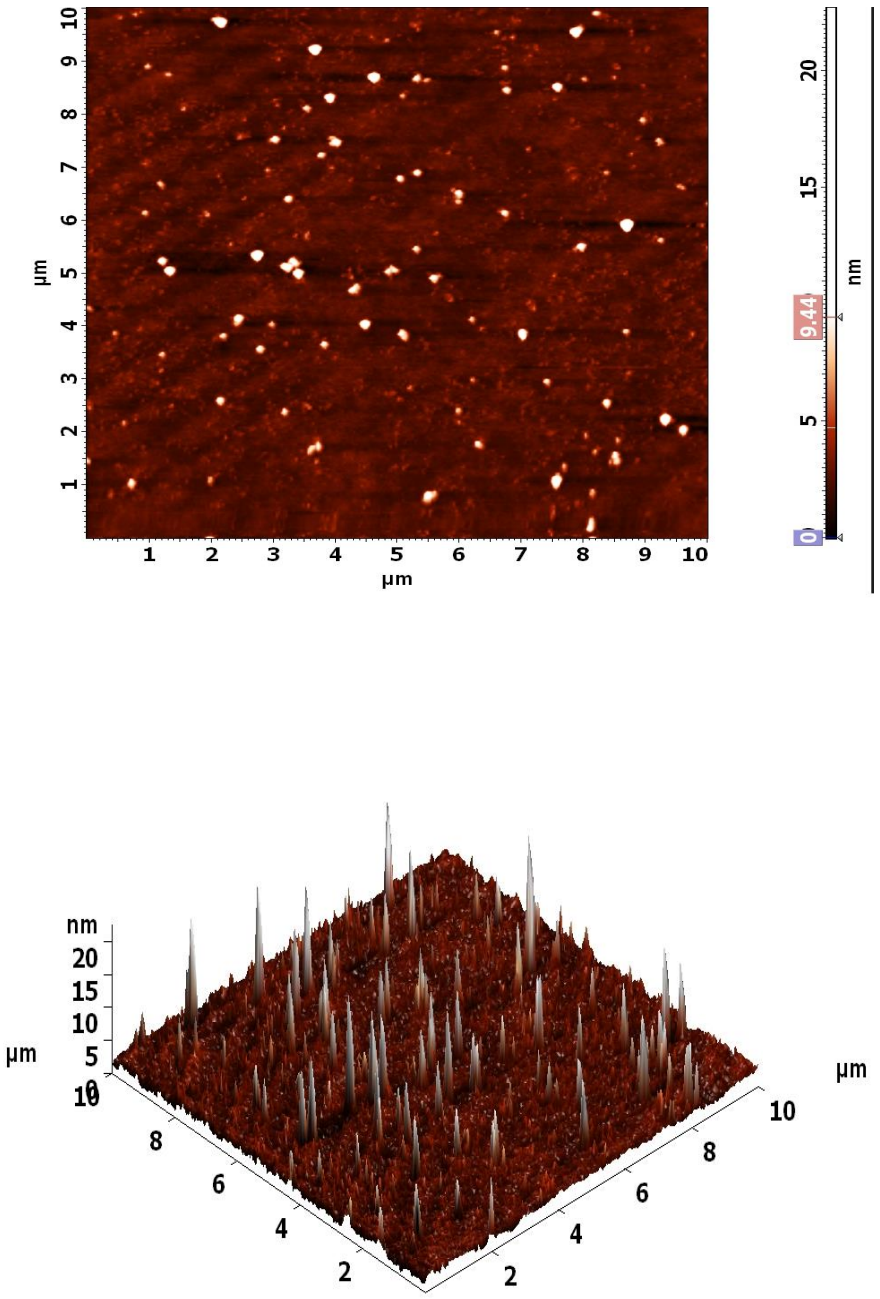


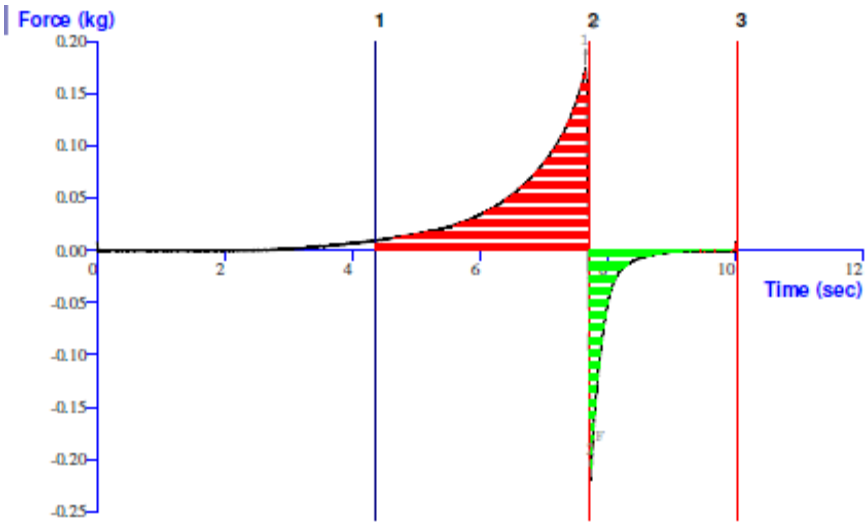
Fig 20:2D image and 3D image of AFM analyzed particle

Atomic Force Microscopy **(**AFM) study results indicate that the formulation has spherical Shape

TEXTURE ANALYSIS

Spreadability:

Spreadability is the ease of which a product can be spread on skin. It is commonly a desired characteristic of ointments, gels, creams and waxes. It is related to the firmness of a product and more often than not the ease of spreading is associated with a loss in firmness. A good gel takes less time to spread and will have high spreadability. During the test the male cone approaches, penetrates and moves into the gel sample for a distance of 25mm from its start point. As the probe penetrates across the gel the force increases until a point of maximum penetration depth. This force value can be taken as the firmness at this specified depth. A firmer sample shows a correspondingly larger area that represents the total amount of force required to perform the shearing process. The probe then proceeds to withdraw from the sample. The maximum negative peak indicates the stickiness of the sample and the maximum negative area is taken as the work of adhesion. A stickier sample will require a greater force to remove the probe, yielding a larger negative area. (Figure no21) represents a typical spredability graph of Niacinamide Cream. The firmness value obtained for Niacinamide Cream (174.393g) with force of application is (169.856g.sec). Similarly the stickiness value for Niacinamide loaded cream is (219.812g).



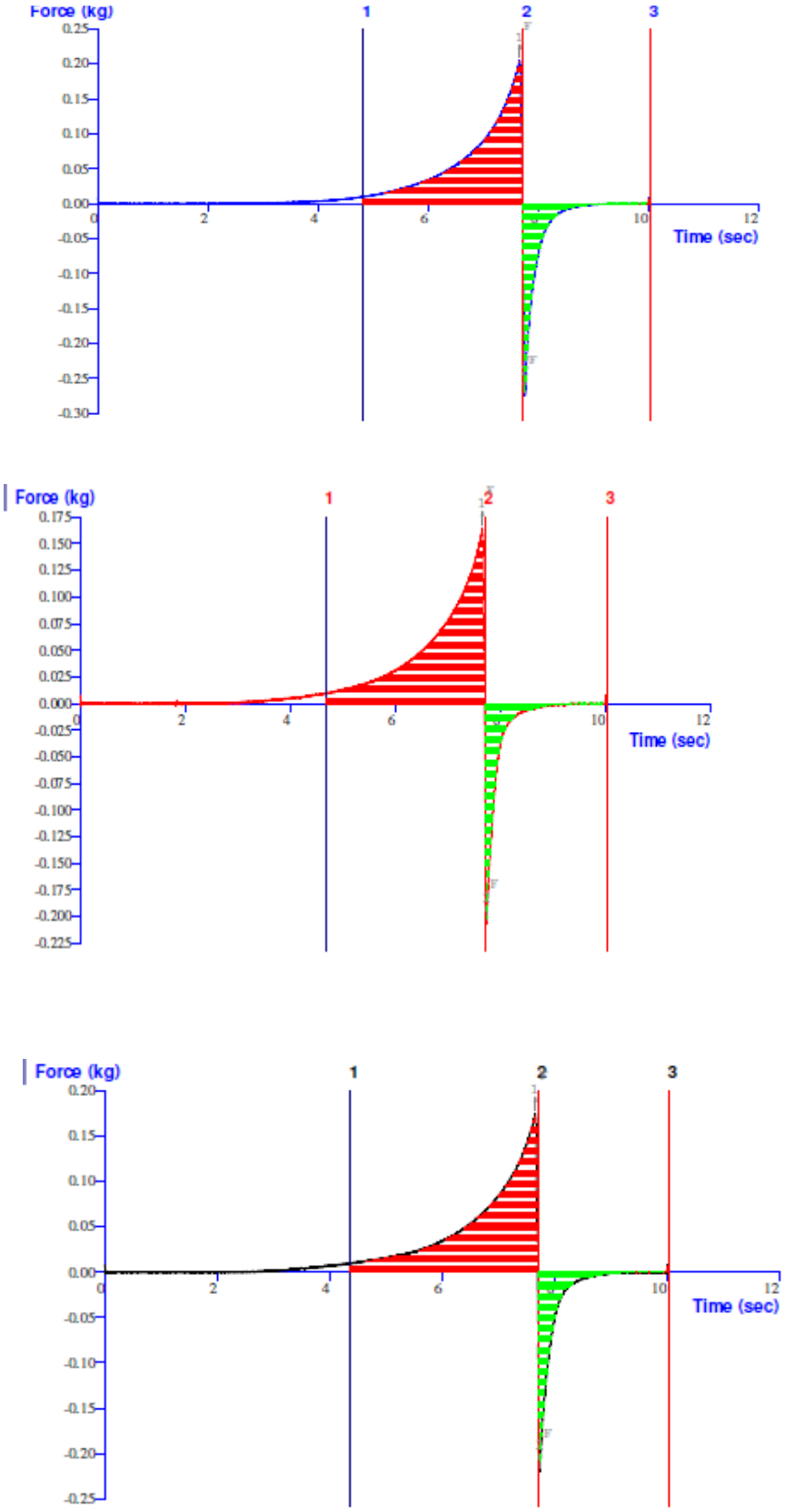
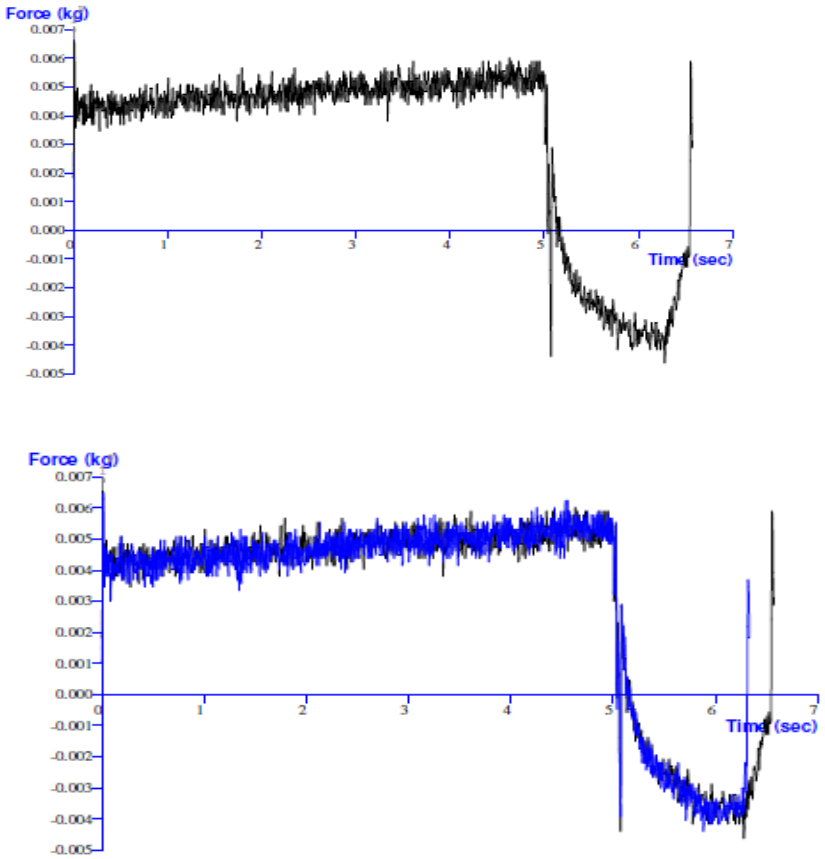


Fig 21: Spreadability plot for Niacinamide loaded Cream

Bloom Strength:

Bloom strength is a measure of the ability of a colloidal dispersion to develop and retain a gel form. It is the force, expressed in grams, necessary to depress by 4 mm the surface of a gel with a standard 0.5" diameter cylinder probe. During the test when a trigger force of 10 g is attained, the probe proceeds to penetrate the cream to a depth of 4 mm. During this penetration the force drops at the point where the cream breaks. After this the resulting forces are due to continuing penetration up to the required depth. The maximum positive force (i.e. the rupture point of the gel) is taken as an indication of rupture strength. The distance that the gel penetrates before this break occurs gives an indication of the gel's elasticity, i.e. a short distance of penetration before break indicates a brittle gel whereas a large distance of penetration before rupture indicates a more elastic gel. A typical bloom strength evaluation plot of Niacinamide loaded cream is shown in (figure no22). The rupture strength value of Niacinamide loaded cream is 6.510g.



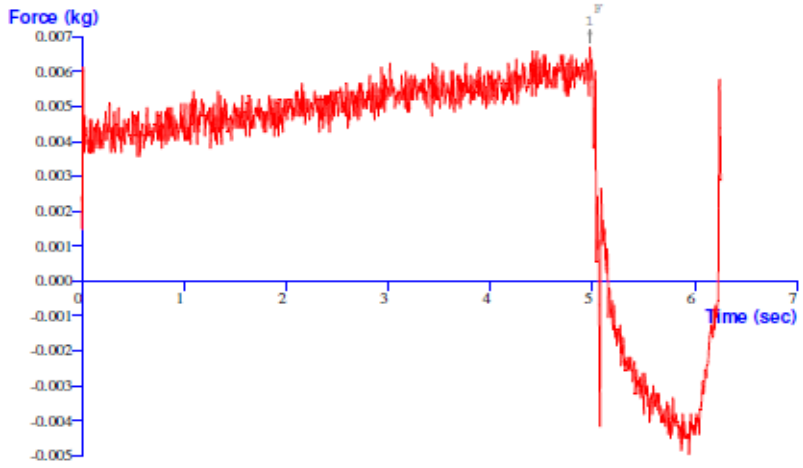


Fig 22: Bloom Strength plot for Niacinamide Loaded Cream

***In Vitro* Drug Release:**

Table 11: *In vitro* Drug Release Study

S.No	**Time (hrs)**	**Cumulative % Drug Release**
1.	2hr	0.75
2.	4hr	4.23
3.	6hr	10.52
4.	8hr	12.78
5.	24hr	52
6.	48hr	92.48

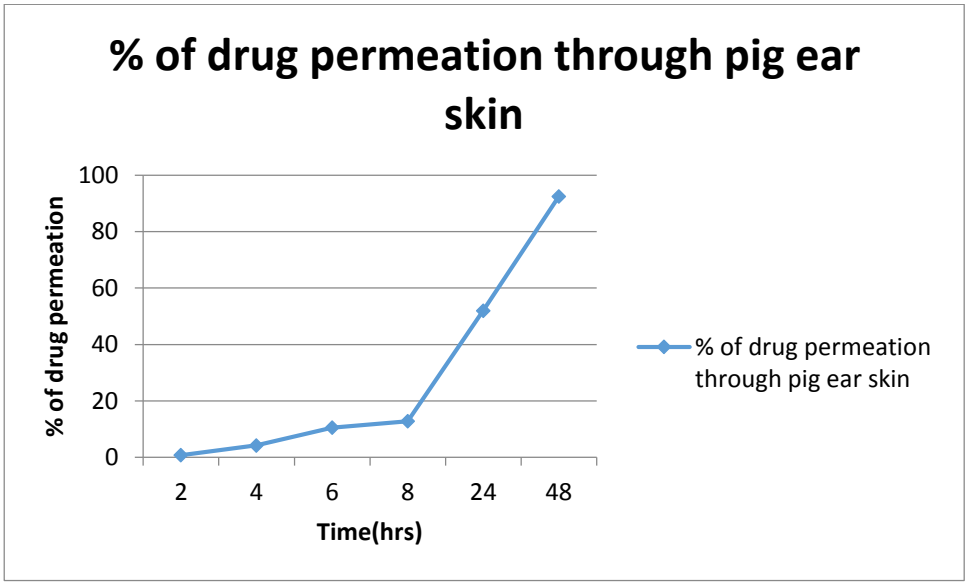


Fig 23: *In vitro* permeation study across pig ear skin

The *in vitro* release studies were carried up to 48 hours for the formulations and 92.42% of drug was penetrated through pig ear skin at 48hrs.

Stability studies

Stability studies of the formulations Niacinamide loaded NLCs cream was carried out for 3 months according to the ICH guidelines. The result showed that there were no significant changes in the cream.

Table12: Stability studies

Formulation Code	1 month	2 month	3 month
NNLC 4 (room temperature)	92.32nm	110.36nm	130.85nm
NNLC 4 (Refrigerator)	85.25nm	90.57nm	96.51nm

SUMMARY AND CONCLUSION

In general lipid based formulations face stability problems over a longer period of time due to microbial contamination which can often change the physical and chemical properties of the drugs and excipients. In the current study, an attempt was made to enhance the stability of Niacinamide loaded lipid based formulations for cosmeceutical purpose using a natural preservation system. Niacinamide loaded nanostructured lipid carriers were prepared for lipid based cream loaded with NLCs of Niacinamide were prepared to serve as a carrier for the delivery of Niacinamide for cosmetic purpose.

Initially, essential oils like cinnamon oil, peppermint oil, eucalyptus oil, lavender oil, clove oil, lemon grass oil, castor oil, neem oil and their mixtures were evaluated for antimicrobial activity. The results indicated a better antimicrobial property for cinnamon oil. Hence, Niacinamide loaded nanostructured lipid carriers and the corresponding creams were prepared using cinnamon oil as liquid oil carrier. Niacinamide loaded NLCs were prepared from compritol, cinnamon oil and saponin by hot homogenization technique using ultra probe sonicator. The spherical shaped nanostructure lipid particles with a particle size of 83 nm showed a sustained release pattern across pig ear skin for around 90% of drug permeation in 48 hours. The prepared NLCs showed good antimicrobial activities with clear zone of inhibition. These Niacinamide loaded nanostructure lipid particles were loaded in a lipid based cream (prepared from cocoa butter, glyceryl monosterate, cinnamon oil, soya lecithin). Evaluation of the texture properties of the lipid cream loaded with Niacinamide NLCs showed good firmness and stickiness. Niacinamide NLCs and lipid based cream loaded with Niacinamide NLCs showed good stability during the initial 3 months without any microbial contamination. Long term stability studies are in progress to evaluate the stability of the lipid based formulations for a period of 1 year.

Lipid based cosmeceuticals prepared using cinnamon oil as a liquid oil can be a good promising natural preservative against microbial contamination and can possibly enhance the stability of several other lipid based cosmeceuticals loaded with different types of drugs.

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